Determining the role of MYC in KRAS-mutant pancreatic cancer

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Abstract

The RAF-MEK-ERK signaling network is the key effector pathway driving KRAS-dependent growth of pancreatic ductal adenocarcinoma (PDAC). We recently demonstrated that ERK is a therapeutic target in PDAC, and that the MYC transcription factor and oncoprotein is a key ERK substrate. PDAC sensitivity to ERK inhibition (ERKi) correlated with loss of MYC. Either KRAS depletion or ERKi resulted in loss of MYC, and MYC suppression alone inhibited PDAC tumorigenic growth. We have initiated a comprehensive evaluation of the specific contributions of MYC to KRAS-dependent PDAC growth. First, RNA-Seq demonstrated that KRAS depletion or ERKi globally suppressed the MYC transcriptome, supporting a significant block in MYC function upon loss of KRAS-ERK signaling. Second, acute *KRAS* suppression or ERKi in both human and mouse PDAC caused striking alterations in cell morphology, with significant cell enlargement and flattening, and enhanced actin stress fiber organization. These changes were largely phenocopied upon MYC suppression. Third, applying reverse phase protein array (RPPA) pathway activation mapping to *KRAS* or *MYC* siRNA-treated PDAC cell lines, we observed alterations in both shared and distinct signaling networks. Loss of either *KRAS* or *MYC* induced compensatory upregulation of KRAS effector signaling, suppressed mitosis, and induced G1 arrest, whereas only *KRAS* depletion activated pro-apoptotic proteins. Additionally, *KRAS* suppression increased E-cadherin whereas MYC suppression reduced it, suggesting opposing consequences on epithelial-to-mesenchymal transition (EMT). Our studies show that KRAS-dependent PDAC growth is mediated through both MYC-dependent and -independent processes. Our ongoing studies involve further evaluation of MYC in KRAS-dependent cellular processes and the use of pharmacologic inhibitors of MYC to further assess MYC as a therapeutic target in KRAS-mutant PDAC.

MYC is essential for ERK-dependent growth

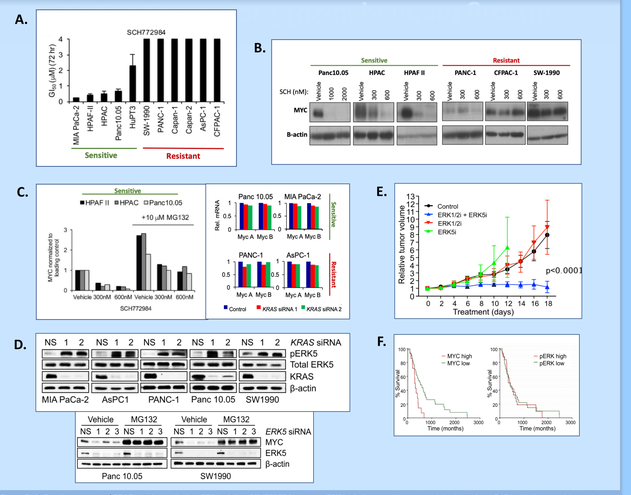

Figure 2. A) Treating a panel of PDAC cell lines with the ERK inhibitor SCH772984 revealed ERK-inhibitor sensitive and resistant cell lines. B) Sensitivity or resistance to ERKi correlated with loss or stabilization of MYC protein, respectively. C) This stabilization of MYC was found to occur at the protein, rather than transcript, level. D) It was found that upon KRAS or ERK inhibition, a compensatory ERK5-dependent mechanism could drive resistance to inhibition via MYC stabilization, underscoring the importance of MYC in PDAC. E) Combining genetic depletion of ERK1/2 and ERK5 in mice showed a remarkably durable tumor response. F) Kaplan-Meier survival plots of PDAC patients show that MYC level, rather than pERK level, correlates with poor prognosis. Data shown are from Hayes et al (2016) and Vaseva et al (2018), Cancer Cell

KRAS and MYC loss cause similar and distinct changes in signaling networks

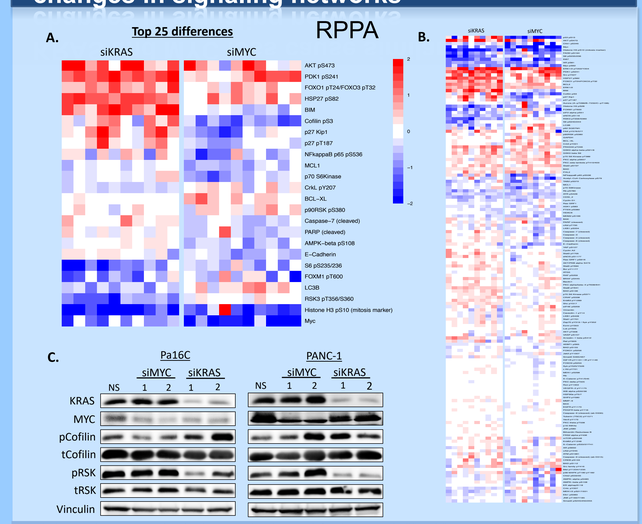

Figure 3. A) Reverse phase protein array (RPPA) analysis was conducted on a panel of KRAS-mutant PDAC cell lines. Cells were treated with KRAS or MYC siRNA to compare the effects of KRAS or MYC loss and begin to dissect the role of MYC in PDAC. Treatment continued for 24 or 72 h (24 h treatment not shown). This figure displays the top 25 differences seen between KRAS and MYC depletion. B) The complete landscape of the 72 h RPPA dataset indicates that KRAS and MYC loss largely display similar consequences. C) Western blots validating a subset of the RPPA data shown in panels A) and B).

Pharmacologic inhibition of MYC suppresses growth

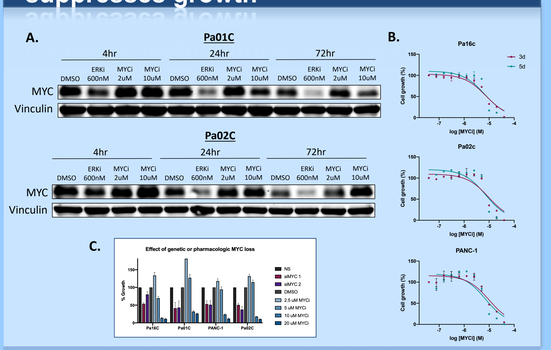

Figure 6. A) Western blots of two PDAC cell lines treated with two concentrations of the MYC inhibitor 975, below and at its GI50 concentration, and an ERK inhibitor positive control. Generally, the MYC inhibitor shows sub-par on-target activity. In Pa01C, the inhibitor depletes some MYC protein; however, in Pa02C MYC protein appears to stabilize even more in response to MYC inhibitor treatment. B) Growth inhibition curves of titrated concentrations of MYC inhibitor in multiple cell lines. C) Comparison of the effect of genetic and pharmacologic inhibition of MYC on cell proliferation.

Conclusions

- MYC is essential for KRAS- and ERK-driven growth of KRAS-mutant PDAC
- MYC is a driver of KRAS-dependent metabolic processes essential for PDAC growth (glycolysis, mitochondrial function, macropinocytosis)
- KRAS and MYC regulate shared and distinct signaling networks
- MYC is essential for KRAS regulation of cell morphology, actin organization, and EMT
- Targeting MYC may be an effective anti-KRAS therapeutic approach

Future Directions

- Application of RNA-Seq to determine the contribution of MYC to the KRAS-regulated transcriptome
- Determine the signaling mechanisms involved in MYC regulation of cell morphology, actin organization and cell migration
- Determine the role of MYC in KRAS regulation of mitochondrial function
- Determine if 'vertical inhibition' of ERK and MYC causes synergistic growth suppression

Acknowledgments

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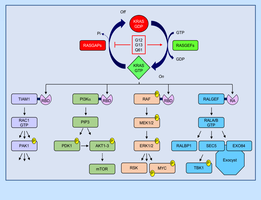
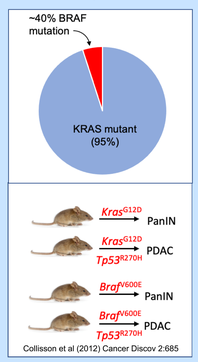
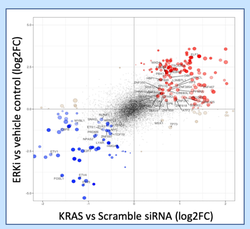
MYC is a critical driver of PDAC growth

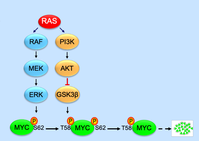
A. Estimated US cancer deaths

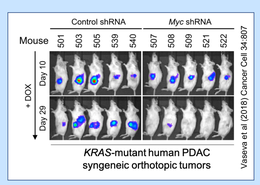
%	Deaths	Site
22.4	135,720	Lung & bronchus
8.8	53,200	Colon & rectum
7.8	47,050	Pancreas
7.0	42,690	Breast
5.5	33,330	Prostate
5.0	30,160	Liver & intrahepatic bile duct
3.3	19,940	Non-Hodgkin lymphoma
3.0	18,020	Brain & nervous system











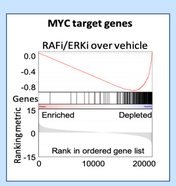


Figure 1. A) Pancreatic ductal adenocarcinoma (PDAC) is a leading cause of cancer deaths in the United States. B) Hyperactivating KRAS mutations, signaling to thousands of effectors, account for over 95% of PDAC cases nationwide. C) One pathway in particular, the RAF-MEK-ERK MAPK cascade, is a key KRAS signaling node. Indicating the importance of RAF, 40% of KRAS-mutant PDAC cases are driven by BRAF mutations, which were shown to phenocopy KRAS in PDAC development in mice. D) The KRAS and ERK transcriptomes display over 86% similarity, indicating the importance of ERK in PDAC tumorigenesis. E) Importantly, RAS can activate and stabilize the oncoprotein MYC through multiple mechanisms, including the MAPK pathway. F) Silencing MYC in KRAS-mutant PDAC tumors resulted in dramatic growth inhibition. E) RAFi and ERKi treatment resulted in drastic reduction of MYC target genes, implicating MAPK signaling in MYC activity and tumorigenesis.

KRAS and MYC genetic depletion phenocopy and cause similar effects on cell growth and morphology

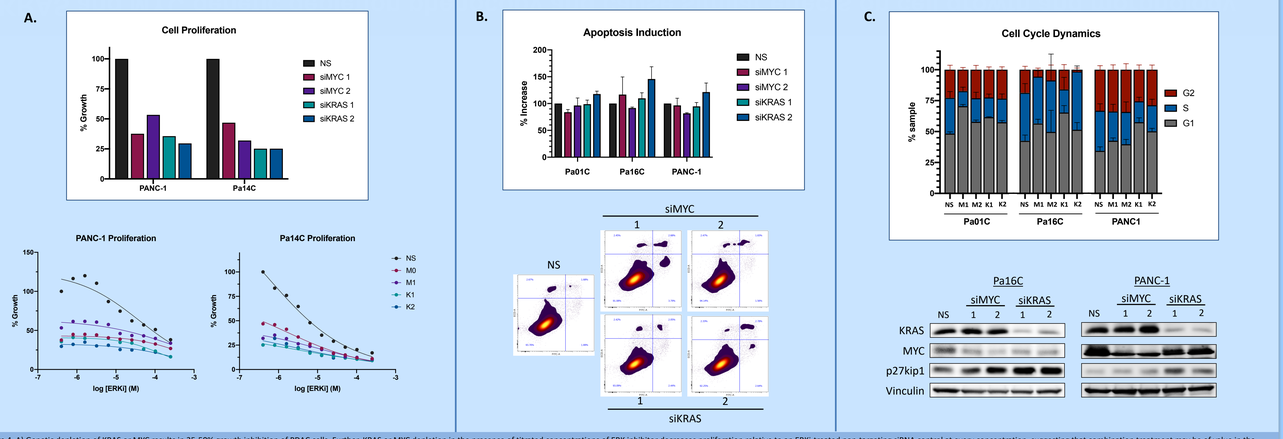


Figure 4. A) Genetic depletion of KRAS or MYC results in 25-50% growth inhibition of PDAC cells. Further, KRAS or MYC depletion in the presence of titrated concentrations of ERK inhibitor decreases proliferation relative to an ERKi-treated non-targeting siRNA control at every concentration, suggesting that combination treatment may be of value in the clinic. B) KRAS or MYC depletion generally does not have a significant effect on apoptosis induction. C) KRAS or MYC depletion causes G1 cell cycle arrest. Both flow cytometry-based assays and Western blots of p27kip1, a marker of G1 arrest, indicate that KRAS loss may induce a more severe arrest phenotype than MYC loss.

KRAS and MYC regulate EMT and cell morphology

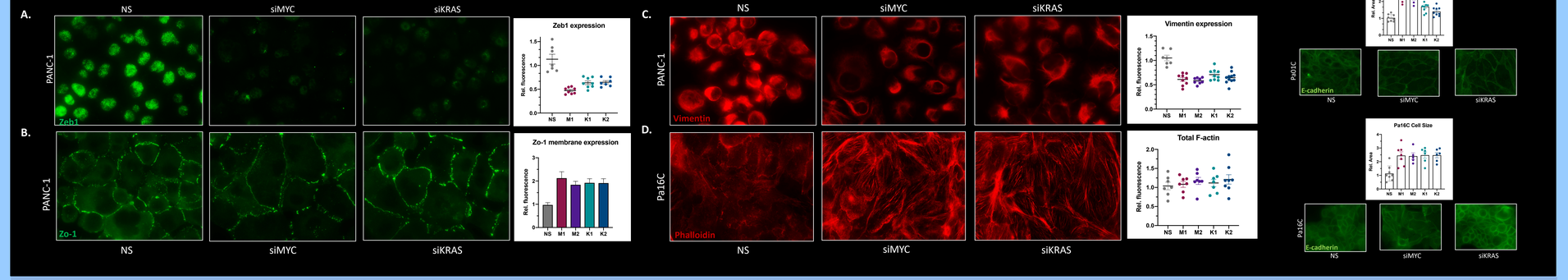


Figure 5. A) Widefield microscopy indicates expression of the mesenchymal marker Zeb1 is reduced with KRAS or MYC genetic depletion. B) Consistent with the findings of panel A), expression of Zo-1, a junctional epithelial marker, increases with KRAS or MYC loss. C) Expression of the mesenchymal marker Vimentin decreases with KRAS and MYC depletion, further supporting an MET transition upon loss of either KRAS or MYC. This is contrary to indications from the RPPA dataset that KRAS and MYC may have opposing consequences on epithelial-to-mesenchymal transition. D) Visualizing F-actin with the cell permeable dye Phalloidin, we observe robust stress fiber formation in KRAS and MYC depleted cells; however, overall F-actin expression does not change. E) In both Pa01C and PA16C cell lines, cell size generally increases upon both KRAS and MYC loss, although the extent to which this effect is observed is variable.