YAP1 overexpression drives resistance to the KRAS^{G12C} specific inhibitor MRTX1257 in KRAS^{G12C}-mutant cancers

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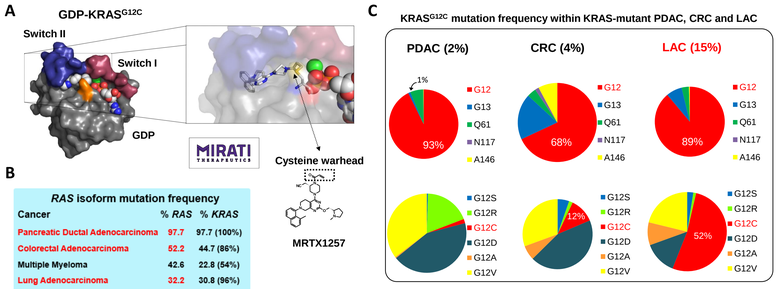
Abstract

Mutations in the KRAS oncogene are among the most frequent driver events in human cancers. With decades of failed efforts, KRAS has been considered 'undruggable'. However, recent discoveries have led to inhibitors that directly bind and inactivate one KRAS mutant, with a glycine-12 to cysteine-12 substitution (G12C), and early clinical evaluation show promising responses in KRAS^{G12C} mutant lung cancers. However, as with essentially all targeted therapies, acquired mechanisms of drug resistance will limit the long-term effectiveness of G12C inhibition (G12Ci). To identify mechanisms of resistance to G12Ci, we determined if overexpression of the HIPPO pathway component, YAP1, can drive resistance to the G12Ci, MRTX1257, an analog of the clinical candidate MRTX849. MRTX1257 is a KRAS^{G12C} mutant-selective covalent inhibitor that demonstrates >1000-fold selectivity over WT KRAS. In a panel of KRAS^{G12C} cell lines, we determined that MRTX1257 potently inhibited KRAS signaling, blocking ERK phosphorylation (IC_{50} ~ 1 nM) and cellular proliferation. Activation of the YAP1 transcriptional co-regulator has been shown to overcome KRAS addiction in KRAS-mutant cancers. In concordance with these observations, we found high YAP1 protein expression significantly correlated with resistance to MRTX1257 in a 3D *in vitro* viability assay. We determined that overexpression of wild-type (WT) YAP1 or constitutively active YAP1^{S127A} (S127A) drove resistance to MRTX1257 in a panel of KRAS^{G12C}-mutant cancer cell lines. YAP1 overexpression also drove resistance to inhibition of ERK MAPK inhibitors, but not other conventional cytotoxic or molecularly targeted chemotherapeutics. Ongoing studies involve the evaluation of targeting YAP1 signaling in combination with G12Ci.

KRAS G12C mutations are prevalent in lung and colorectal cancers

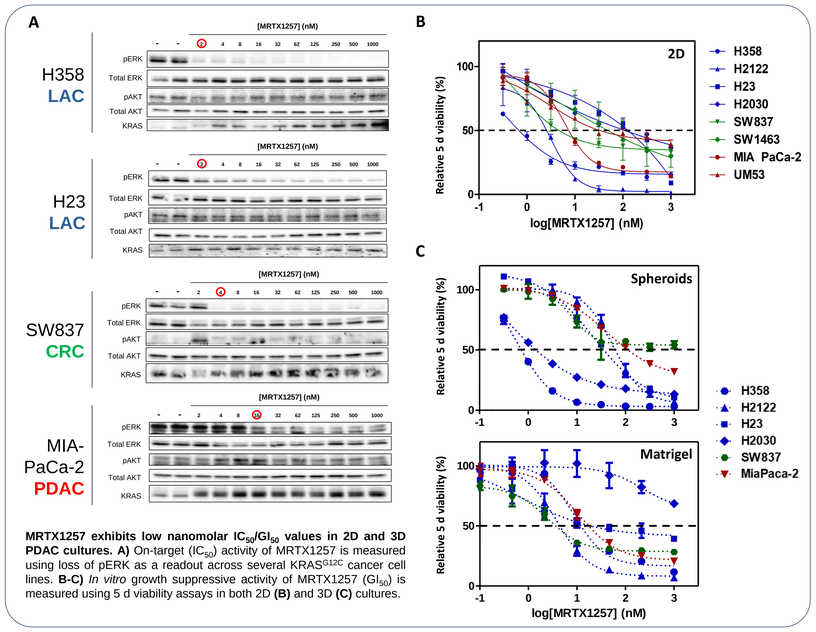


Estimated US cancer deaths		
Site	Deaths	%
Lung & bronchus	135,720	22.4
Colon & rectum	53,200	8.8
Pancreas	47,050	7.8

G12Ci covalent modification of the C12 residue in mutant KRAS^{G12C} locks it into its inactive, GDP-bound state. A) Crystal structure showing GDP-KRAS^{G12C} covalently bound by MRTX1257 at C12 in the S-IIP; thereby locking it in its inactive state **B)** *RAS* (and *KRAS*) mutational frequency across the three most deadly forms of human cancer, pancreatic (PDAC) colorectal (CRC) and lung adenocarcinoma (LAC). **C)** Distribution of KRAS-G12C mutations within KRAS-mutant PDAC, CRC and LAC

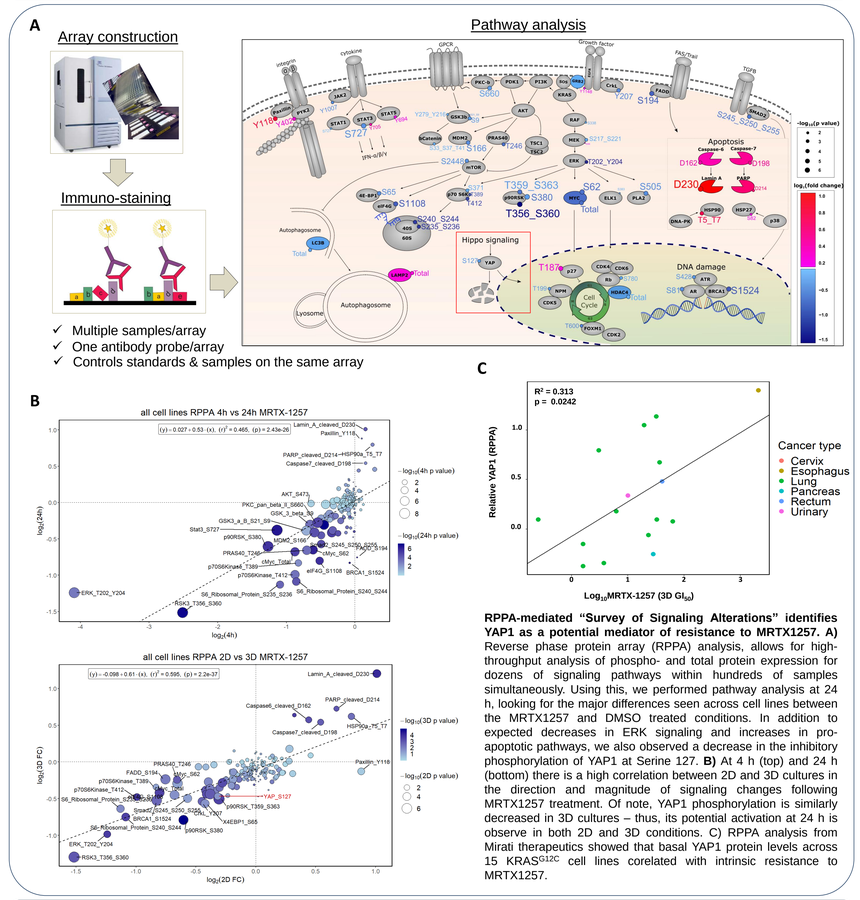
*COSMIC v88; **Cox et al (2014) *Nat Rev Drug Discov* 13:828; Siegel et al (2020) CA Cancer J Clin 70:9 F; ell JB, et al. (2018) ACS Med Chem Lett 9(12):1230-1234

MRTX1257 is a potent KRAS^{G12C} inhibitor



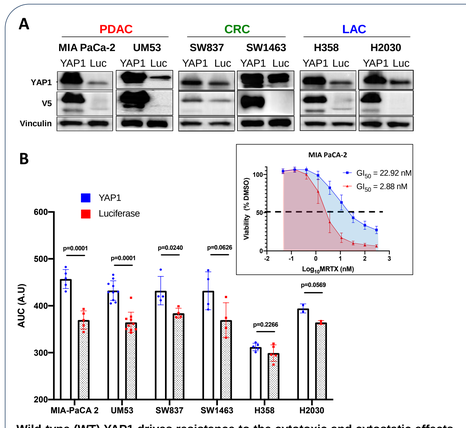
MRTX1257 exhibits low nanomolar IC_{50}/GI_{50} values in 2D and 3D PDAC cultures. A) On-target (IC_{50}) activity of MRTX1257 is measured using loss of pERK as a readout across several KRAS^{G12C} cancer cell lines. **B-C)** *In vitro* growth suppressive activity of MRTX1257 (GI_{50}) is measured using 5 d viability assays in both 2D **(B)** and 3D **(C)** cultures.

RPPA analysis identifies YAP1 activation as a potential mechanism of resistance to the loss of KRAS^{G12C}



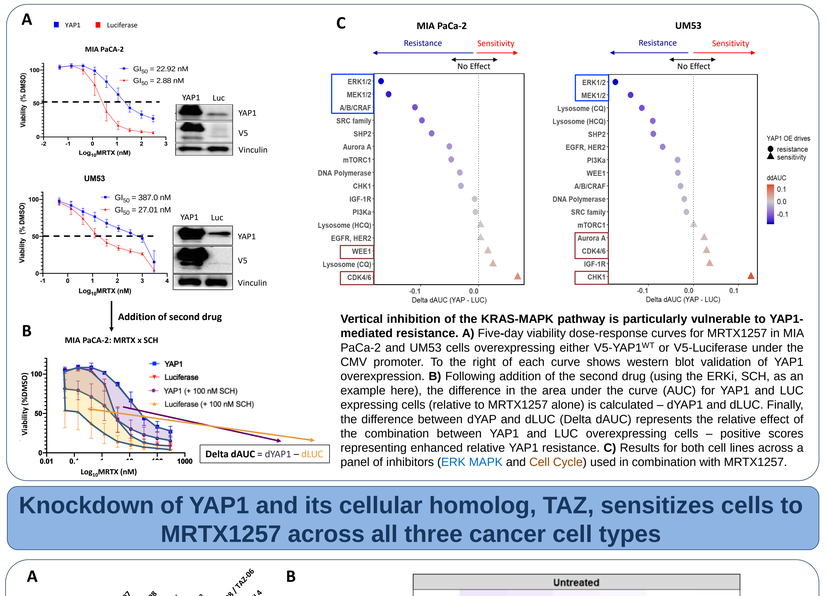
RPPA-mediated "Survey of Signaling Alterations" identifies YAP1 as a potential mediator of resistance to MRTX1257. A) Reverse phase protein array (RPPA) analysis, allows for high-throughput analysis of phospho- and total protein expression for dozens of signaling pathways within hundreds of samples simultaneously. Using this, we performed pathway analysis at 24 h, looking for the major differences seen across cell lines between the MRTX1257 and DMSO treated conditions. In addition to expected decreases in ERK signaling and increases in pro-apoptotic pathways, we also observed a decrease in the inhibitory phosphorylation of YAP1 at Serine 127. **B)** At 4 h (top) and 24 h (bottom) there is a high correlation between 2D and 3D cultures in the direction and magnitude of signaling changes following MRTX1257 treatment. Of note, YAP1 phosphorylation is similarly decreased in 3D cultures – thus, its potential activation at 24 h is observe in both 2D and 3D conditions. C) RPPA analysis from Mirati therapeutics showed that basal YAP1 protein levels across 15 KRAS^{G12C} cell lines corelated with intrinsic resistance to MRTX1257.

YAP1 activation drives resistance to MRTX1257 in 2D viability assays



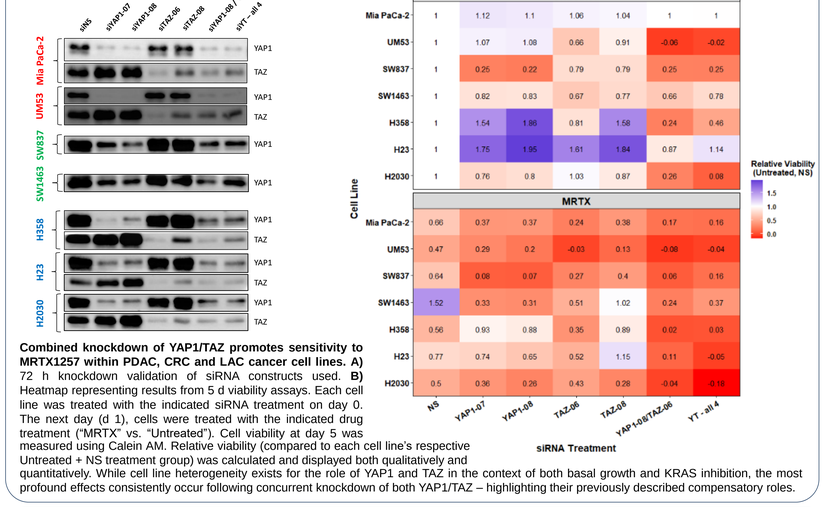
Wild-type (WT) YAP1 drives resistance to the cytotoxic and cytostatic effects of MRTX1257. A) Western blot validation of V5-tagged YAP1-WT overexpression in all six KRAS^{G12C} cell lines tested. *Note*: YAP1 expression is under control of the CMV promoter and therefore constitutively expressed. **B)** Five-day viability assays with MRTX1257 were performed in YAP1 and Luciferase cell lines. In order to adequately gauge the effect of YAP1 over the entire dose-response curve, area under the curve analysis (AUC) was performed (top right panel, example in MIA PaCa-2 cells). Similar analysis was performed in all six cells lines. While YAP1 was able to promote resistance to MRTX1257 in PDAC and CRC cells, this response was largely unseen in the two LAC cell lines tested. **C)** Five-day apoptosis assay using Annexin-FITC (early apotposis) and Propidium idodide (late apoptosis) – note, only representative data from MIA PaCa-2 using 100 nM MRTX is shown here. **D)** Cumulative apoptosis data taken for both PDAC cell lines – YAP1 overexpression mitigates the dose-dependent cytotoxic effects of MRTX1257 in these cells.

YAP1 activation drives preferential resistance to MRTX1257 in combination with ERK MAPK inhibitors



Vertical inhibition of the KRAS-MAPK pathway is particularly vulnerable to YAP1-mediated resistance. A) Five-day viability dose-response curves for MRTX1257 in MIA PaCa-2 and UM53 cells overexpressing either V5-YAP1^{WT} or V5-Luciferase under the CMV promoter. To the right of each curve shows western blot validation of YAP1 overexpression. **B)** Following addition of the second drug (using the ERKi, SCH, as an example here), the difference in the area under the curve (AUC) for YAP1 and LUC expressing cells (relative to MRTX1257 alone) is calculated – dYAP1 and dLUC. Finally, the difference between dYAP and dLUC (Delta dAUC) represents the relative effect of the combination between YAP1 and LUC overexpressing cells – positive scores representing enhanced relative YAP1 resistance. **C)** Results for both cell lines across a panel of inhibitors (ERK MAPK and Cell Cycle) used in combination with MRTX1257.

Knockdown of YAP1 and its cellular homolog, TAZ, sensitizes cells to MRTX1257 across all three cancer cell types



Combined knockdown of YAP1/TAZ promotes sensitivity to MRTX1257 within PDAC, CRC and LAC cancer cell lines. A) 72 h knockdown validation of siRNA constructs used. **B)** Heatmap representing results from 5 d viability assays. Each cell line was treated with the indicated siRNA treatment on day 0. The next day (d 1), cells were treated with the indicated drug treatment ("MRTX" vs. "Untreated"). Cell viability at day 5 was measured using Calein AM. Relative viability (compared to each cell line's respective Untreated + NS treatment group) was calculated and displayed both qualitatively and quantitatively. While cell line heterogeneity exists for the role of YAP1 and TAZ in the context of both basal growth and KRAS inhibition, the most profound effects consistently occur following concurrent knockdown of both YAP1/TAZ – highlighting their previously described compensatory roles.

Conclusions/Future Directions

- YAP1-WT overexpression enables bypass of KRAS addiction in a subset of KRAS G12C mutant cancer cell lines
- MRTX1257 in combination with additional ERK MAPK pathway inhibitors are most vulnerable to YAP1-mediated resistance, while cell cycle inhibitors appear most capable of mitigating this resistance
- YAP1 and TAZ compensate for each other in the context of oncogenic KRAS. Targeting them concurrently sensitizes cells to G12Ci
- Determine which transcription factors YAP1/TAZ are using to mediate resistance to G12Ci, with an initial focus on the TEAD family, AP1 and MYC
- Because YAP1/TAZ are currently undruggable, determine alternative ways to target them. For example, targeting the TEAD transcription factors or mediators of actin polymerization, a process known to activate YAP1/TAZ

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