Stromal reprogramming overcomes resistance to RAS/MEK inhibition to improve pancreas cancer responses to cytotoxic therapy

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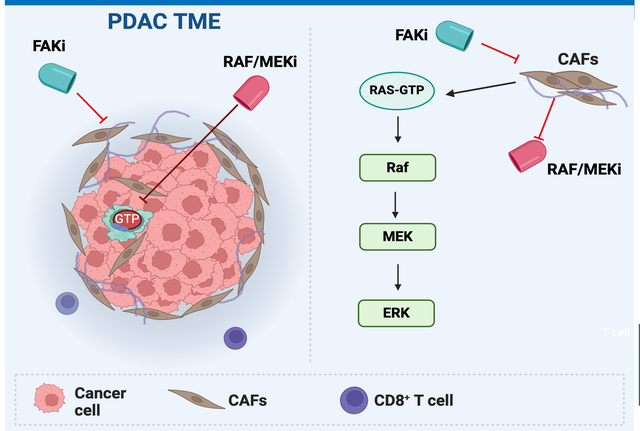
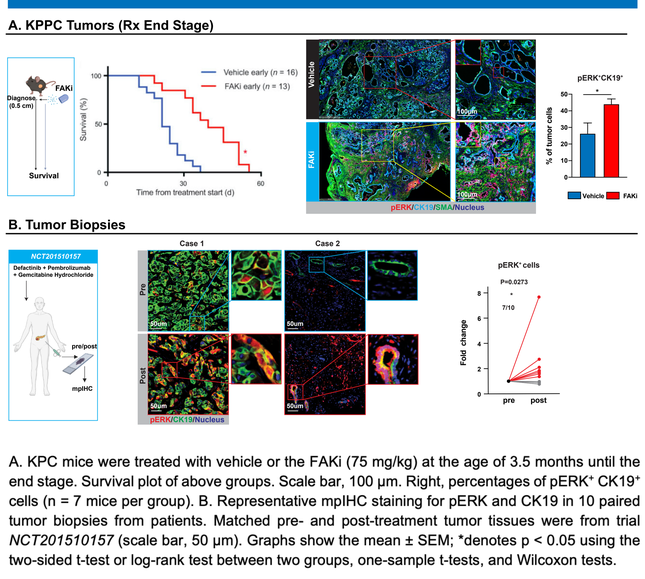
Abstract

Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal malignancy with a poor response rate to therapy. An immune suppressive tumor microenvironment (TME) and oncogenic mutations in KRAS have been implicated as drivers of resistance to both conventional and immune therapies. As such, targeting RAS/MAPK signaling is an attractive strategy. However, RAS/MAPK inhibition has not yet shown clinical efficacy for PDAC, likely due to the rapid acquisition of resistance in PDAC cells. Tumor intrinsic mechanisms of resistance to RAS/MAPK have been studied, however, the unique PDAC TME may also be a key driver in resistance. Previous studies have shown that FAK inhibition can reprogram the PDAC TME and delay PDAC progression in animal models. Herein, we found that long-term FAK inhibitor treatment led to hyperactivation of the RAS/MAPK pathway in both genetically engineered mouse models and in post-treatment PDAC tissues from FAK inhibitor clinical trials. Concomitant inhibition of both FAK (VS-4718) and RAF/MEK (avutometinib) signaling dramatically suppressed tumor growth, leading to increased survival across multiple PDAC mouse models. The mechanisms of synergy include both changes in tumor-intrinsic signaling and modulation of tumor/stroma interactions that drive avutometinib resistance. In the TME, we found that cancer-associated fibroblasts (CAFs) can impair the downregulation of cMyc by RAF/MEK inhibition in PDAC cells. This resulted in de-novo resistance to RAF/MEK inhibition in fibrotic conditions. By contrast, FAK inhibitors reprogramed CAFs to suppress the production of key growth factors, including FGF1, that drove resistance to RAF/MEK inhibition. While combined FAK and RAF/MEK inhibition only led to disease stasis, the addition of chemotherapy to the combination led to tumor regression and improved long-term survival in PDAC mouse models. Analysis of tumor immunity showed that the combination of FAK and RAF/MEK inhibition improved anti-tumor immunity and improved priming of T cell responses, which was further improved with the addition of chemotherapy. These findings led to the testing of FAK (defactinib) plus RAF/MEK (avutometinib) inhibition in combination with gemcitabine and nab-paclitaxel in advanced front-line pancreatic cancer patients (RAMP 205; NCT05669482). Finally, we tested whether the addition of immunotherapy could enhance the efficacy of FAKi + RAF/MEKi + chemotherapy and found that addition of either PD-1 or CTLA4/PD-1 blockade led to long-term disease control in PDAC animal models. Together, these studies identified FAK inhibition as a novel approach to overcome both tumor intrinsic and stromal-derived resistance to RAS/MAPK inhibition and showed that this combination can be exploited to increase the efficacy of cytotoxic and immunotherapy approaches.

Introduction

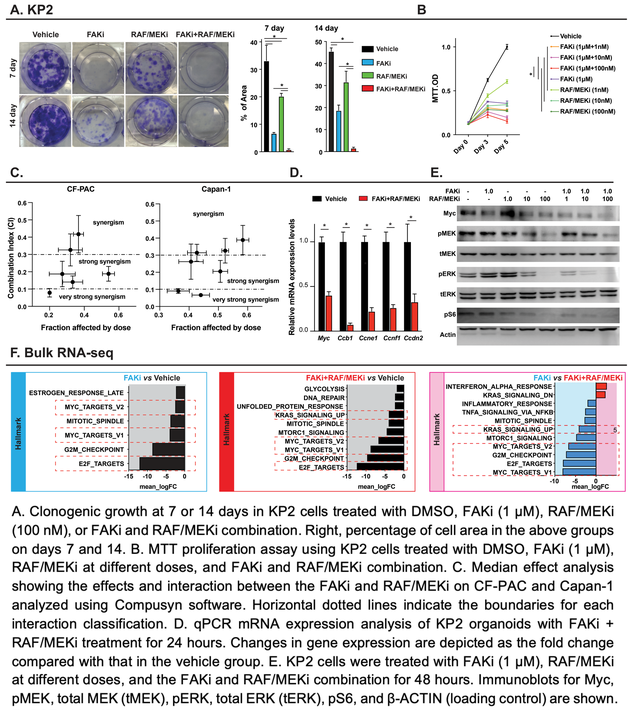


MAPK hyperactivation in FAKi-treated PDAC tumors



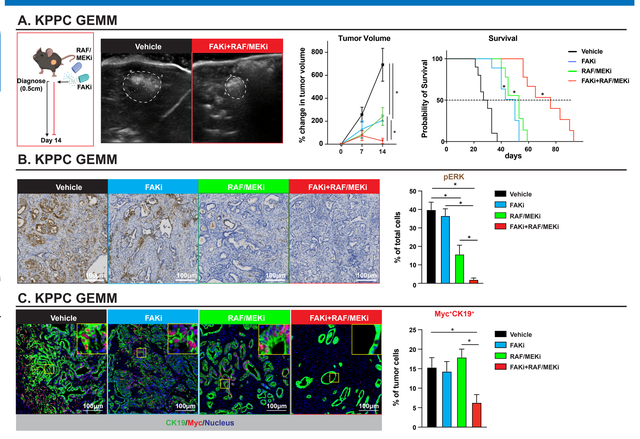
A. KPC mice were treated with vehicle or the FAKi (75 mg/kg) at the age of 3.5 months until the end stage. Survival plot of above groups. Scale bar, 100 µm. Right, percentages of pERK$^+$ CK19$^+$ cells (n = 7 mice per group). B. Representative mpIHC staining for pERK and CK19 in 10 paired tumor biopsies from patients. Matched pre- and post-treatment tumor tissues were from trial *NCT201510157* (scale bar, 50 µm). Graphs show the mean ± SEM; *denotes p < 0.05 using the two-sided t-test or log-rank test between two groups, one-sample t-tests, and Wilcoxon tests.

RAS/MEK inhibition synergizes with FAK inhibition by inhibiting downstream Myc expression.



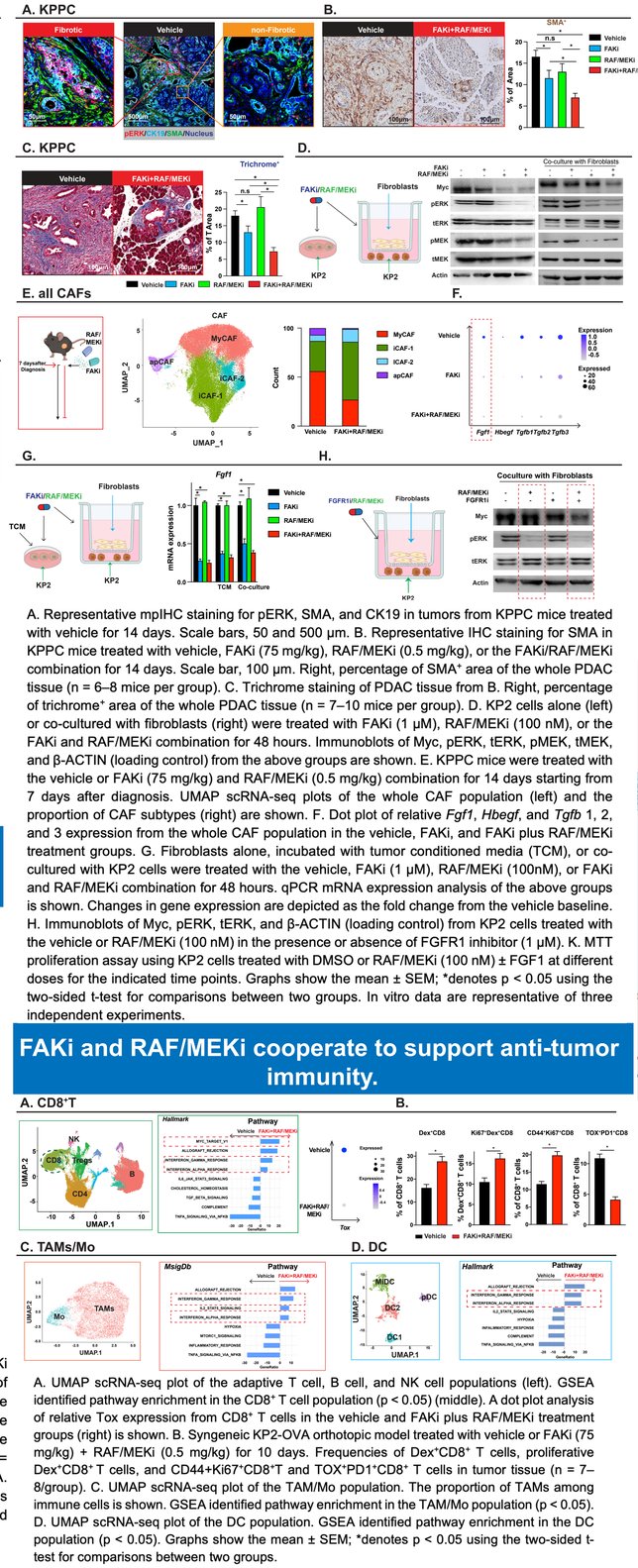
A. Clonogenic growth at 7 or 14 days in KP2 cells treated with DMSO, FAKi (1 µM), RAF/MEKi (100 nM), or FAKi and RAF/MEKi combination. Right, percentage of cell area in the above groups on days 7 and 14. B. MTT proliferation assay using KP2 cells treated with DMSO, FAKi (1 µM), RAF/MEKi at different doses, and FAKi and RAF/MEKi combination. C. Median effect analysis showing the effects and interaction between the FAKi and RAF/MEKi on CF-PAC and Capan-1 analyzed using Compusyn software. Horizontal dotted lines indicate the boundaries for each interaction classification. D. qPCR mRNA expression analysis of KP2 organoids with FAKi + RAF/MEKi treatment for 24 hours. Changes in gene expression are depicted as the fold change compared with that in the vehicle group. E. KP2 cells were treated with FAKi (1 µM), RAF/MEKi at different doses, and the FAKi and RAF/MEKi combination for 48 hours. Immunoblots for Myc, pMEK, total MEK (tMEK), pERK, total ERK (tERK), pS6, and β-ACTIN (loading control) are shown. F. GSEA identified pathway enrichment in KP2 organoids treated with FAKi (1 µM, left) and FAKi + RAF/MEKi (100 nM, middle) for 24 hours, compared with the vehicle group. Right, GSEA identified pathway enrichment in FAKi-treated KP2 organoids compared with that in FAKi + RAF/MEKi-treated samples. Graphs show the mean ± SEM; *denotes p < 0.05 using the two-sided t-test for comparisons between two groups. In vitro data are representative of three independent experiments.

FAK/RAF/MEK inhibition blocks RAS/MAPK/Myc signaling and delays PDAC progression.



A. KPPC mice were treated with vehicle, FAKi (75 mg/kg), RAF/MEKi (0.5 mg/kg), and the FAKi and RAF/MEKi combination for 14 days after diagnosis. Representative ultrasound images of tumors on day 14 from the vehicle and combination groups are shown. The mean percent change in tumor volume on days 7 and 14 from ultrasound measurements is shown (n = 8–13). The Kaplan–Meier survival analysis of the above groups is shown (n = 10/group). B. Representative IHC staining for pERK in tissue from A. Scale bar, 100 µm. Right, percentage of pERK$^+$ cells (n = 7–8 mice per group). C. Representative mpIHC staining for Myc and CK19 in tumors from A. Scale bar, 100 µm. Right, percentages of Myc$^+$ CK19$^+$ cells (among CK19$^+$ cells) and Myc+ cells (n = 7–8 mice per group). Graphs show the mean ± SEM; *denotes p < 0.05 using the two-sided t-test or log-rank test for comparisons between two groups.

FAKi-induced reduction of FGF1 in CAFs synergized with RAF/MEKi to decrease Myc

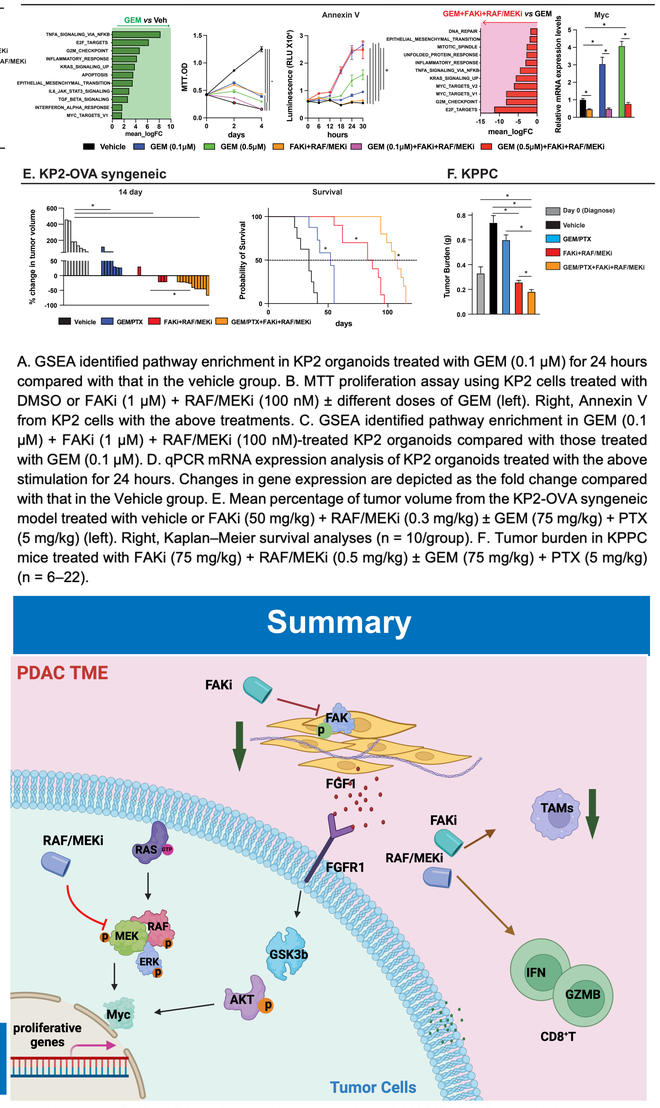


A. Representative mpIHC staining for pERK, SMA, and CK19 in tumors from KPPC mice treated with vehicle for 14 days. Scale bars, 50 and 500 µm. B. Representative IHC staining for SMA in KPPC mice treated with vehicle, FAKi (75 mg/kg), RAF/MEKi (0.5 mg/kg), or the FAKi/RAF/MEKi combination for 14 days. Scale bar, 100 µm. Right, percentage of SMA$^+$ area of the whole PDAC tissue (n = 6–8 mice per group). C. Trichrome staining of PDAC tissue from B. Right, percentage of trichrome$^+$ area of the whole PDAC tissue (n = 7–10 mice per group). D. KP2 cells alone (left) or co-cultured with fibroblasts (right) were treated with FAKi (1 µM), RAF/MEKi (100 nM), or the FAKi and RAF/MEKi combination for 48 hours. Immunoblots of Myc, pERK, tERK, pMEK, tMEK, and β-ACTIN (loading control) from the above groups are shown. E. KPPC mice were treated with the vehicle or FAKi (75 mg/kg) and RAF/MEKi (0.5 mg/kg) combination for 14 days starting from 7 days after diagnosis. UMAP scRNA-seq plots of the whole CAF population (left) and the proportion of CAF subtypes (right) are shown. F. Dot plot of relative *Fgf1*, *Hbegf*, and *Tgfb* 1, 2, and 3 expression from the whole CAF population in the vehicle, FAKi, and FAKi plus RAF/MEKi treatment groups. G. Fibroblasts alone, incubated with tumor conditioned media (TCM), or co-cultured with KP2 cells were treated with the vehicle, FAKi (1 µM), RAF/MEKi (100nM), or FAKi and RAF/MEKi combination for 48 hours. qPCR mRNA expression analysis of the above groups is shown. Changes in gene expression are depicted as the fold change from the vehicle baseline. H. Immunoblots of Myc, pERK, tERK, and β-ACTIN (loading control) from KP2 cells treated with the vehicle or RAF/MEKi (100 nM) in the presence or absence of FGFR1 inhibitor (1 µM). K. MTT proliferation assay using KP2 cells treated with DMSO or RAF/MEKi (100 nM) ± FGF1 at different doses for the indicated time points. Graphs show the mean ± SEM; *denotes p < 0.05 using the two-sided t-test for comparisons between two groups. In vitro data are representative of three independent experiments.

FAKi and RAF/MEKi cooperate to support anti-tumor immunity.

A. UMAP scRNA-seq plot of the adaptive T cell, B cell, and NK cell populations (left). GSEA identified pathway enrichment in the CD8$^+$ T cell population (p < 0.05) (middle). A dot plot analysis of relative Tox expression from CD8$^+$ T cells in the vehicle and FAKi plus RAF/MEKi treatment groups (right) is shown. B. Syngeneic KP2-OVA orthotopic model treated with vehicle or FAKi (75 mg/kg) + RAF/MEKi (0.5 mg/kg) for 10 days. Frequencies of Dex$^+$CD8$^+$ T cells, proliferative Dex$^+$CD8$^+$ T cells, and CD44+Ki67$^+$CD8$^+$T and TOX$^+$PD1$^+$CD8$^+$ T cells in tumor tissue (n = 7–8/group). C. UMAP scRNA-seq plot of the TAM/Mo population. The proportion of TAMs among immune cells is shown. GSEA identified pathway enrichment in the TAM/Mo population (p < 0.05). D. UMAP scRNA-seq plot of the DC population. GSEA identified pathway enrichment in the DC population (p < 0.05). Graphs show the mean ± SEM; *denotes p < 0.05 using the two-sided t-test for comparisons between two groups.

FAKi combined with RAF/MEKi treatment improves the chemotherapy response in PDAC



A. GSEA identified pathway enrichment in KP2 organoids treated with GEM (0.1 µM) for 24 hours compared with that in the vehicle group. B. MTT proliferation assay using KP2 cells treated with DMSO or FAKi (1 µM) + RAF/MEKi (100 nM) ± different doses of GEM (left). Right, Annexin V from KP2 cells with the above treatments. C. GSEA identified pathway enrichment in GEM (0.1 µM) + FAKi (1 µM) + RAF/MEKi (100 nM)-treated KP2 organoids compared with those treated with GEM (0.1 µM). D. qPCR mRNA expression analysis of KP2 organoids treated with the above stimulation for 24 hours. Changes in gene expression are depicted as the fold change compared with that in the Vehicle group. E. Mean percentage of tumor volume from the KP2-OVA syngeneic model treated with vehicle or FAKi (50 mg/kg) + RAF/MEKi (0.3 mg/kg) ± GEM (75 mg/kg) + PTX (5 mg/kg) (left). Right, Kaplan–Meier survival analyses (n = 10/group). F. Tumor burden in KPPC mice treated with FAKi (75 mg/kg) + RAF/MEKi (0.5 mg/kg) ± GEM (75 mg/kg) + PTX (5 mg/kg) (n = 6–22).

Summary

1. Long-term FAK inhibition induces hyperactivation of MAPK signaling in both preclinical models and in tumors from patients.
2. Dual inhibition of FAK and RAF/MEK induces tumor growth inhibition and increased survival relative to single agents.
3. FAK blockade reprograms cancer-associated fibroblasts to synergize with RAF/MEK inhibition.
4. The addition of FAK and RAF/MEK inhibitors to chemotherapy induces strong tumor regressions and improved survival relative to chemotherapy alone.
5. These results support the ongoing clinical study of avutometinib (RAF/MEK clamp) and defactinib (FAK inhibitor) in combination with standard-of-care chemotherapy (gemcitabine/nab-paclitaxel) for patients with previously untreated metastatic pancreas cancer (RAMP 205; NCT05669482)

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