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THE ROLE OF P21-ACTIVATED KINASE 4 (PAK4) IN CANCER STEMNESS AND EPITHELIAL-TO-MESENCHYMAL TRANSITION

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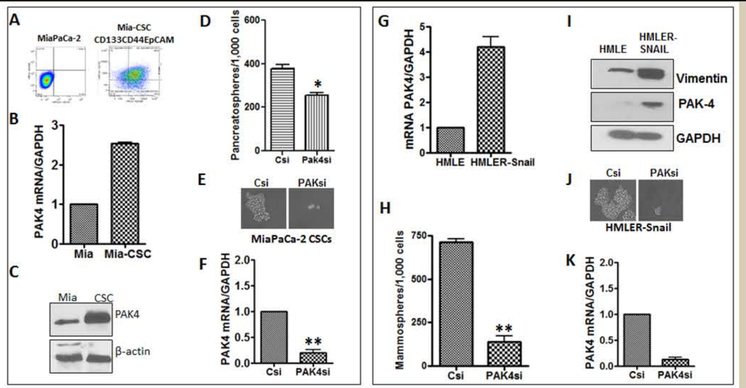
ABSTRACT

PAK4 is a key downstream effector of the Rho family GTPases and is found to be over-expressed in many oncogenic Ras-driven cancers. Mesenchymal pancreatic ductal adenocarcinoma (PDAC) stem cells (CSCs) that are triple positive for stemness markers (CD133+, CD44+, and EpCAM+) as well as Ras and snail transduced human mammary epithelial cells (HMLER-snail) showed enhanced expression of PAK4 along with activation of Rho, Rac1 and CDC42. This makes PAK4 and attrative target against EMT and stem cells. Here we evaluate the impact of PAK4 inhibition on PDAC and HMLER-Snail CSC models.

METHODS

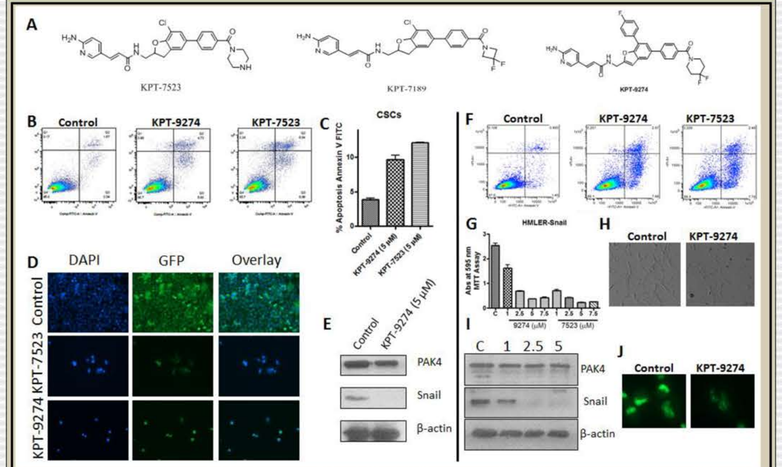
PDAC CSCs were isolated by flow sorting for CD133CD44EpCAM^{+++}, HMECs that are transduced with Ras and the EMT promoter snail (HMLER-Snail) were obtained from Dr. Robert Weinberg at Whitehead Institute. PAK4 allosteric modulators [PAMs [KPT-7189, KPT-7523 and KPT-9274] were evaluated for their impact on EMT and stemness in vitro and in CSC derived sub-cutaneous xenografts.

RESULTS

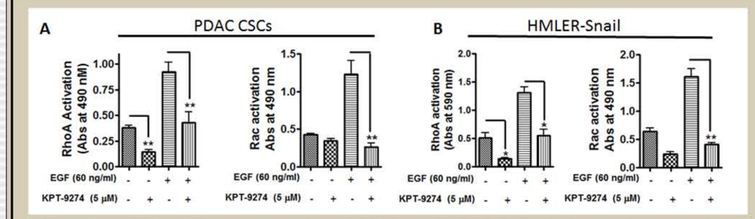


PAK4 expression is directly correlated to stemness and mesenchymal characteristics. Relative expression of PAK4 mRNA and protein **[Left A-C]** MiaPaCa-2 vs MiaPaCa-2-CSC and **[Right G-I]** HMLE vs HMLER-Snail cells. PAK4 siRNA silencing abrogates spheroid formation of MiaPaCa-2-CSC **[D-F]** and HMLER-Snail cells **[J-K].**

RESULTS

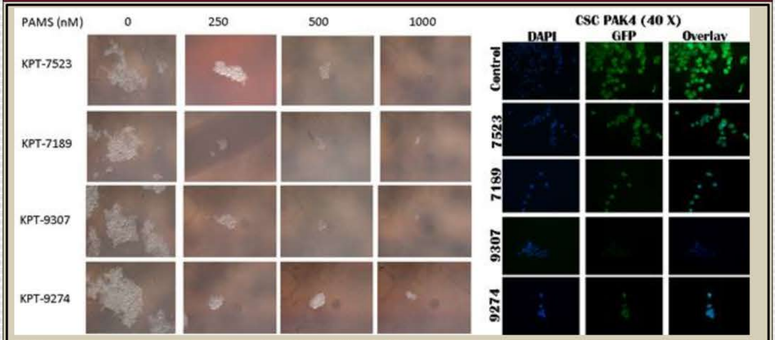


Targeting PAK4 suppresses EMT in stem cell models. **[A]** Structures of PAK4 allosteric moldulators. [**B and C**] MiaPaCa-2-CSCs were grown in DMEM in six well plates (50,000 cells/well) and exposed to 5µM concentrations of PAMs for 72 hrs followed by Annexin V FITC apoptosis analysis. [**D**] CSCs and HMLER-Snail cells were grown in chambered slides (3000 cells/chamber) and exposed to 5 µM concentration of indicated PAMs for 72 hrs. Immunofluorescence assay was performed using Snail antibody. **[E]** Western blotting under similar conditions showing down-regulation of Snail. **[F]** Annexin Apoptosis analysis in HMLER-Snail cells (dosing as above). [G] MTT assay (5000 cells per well in 96 well plate and exposed to PAMs for 72 hrs). [**H**] PAMs reverse EMT morphology of HMLER-Snail and suppress snail **[J]** IF analysis showing snail suppression in HMLER-Snail cells.

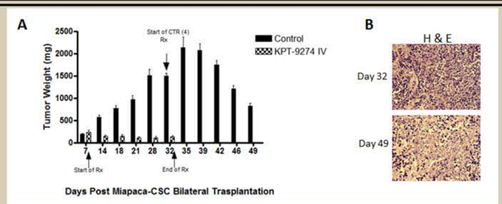


PAMs suppress Rho and Rac activation. [A and B] CSCs or HMLER-Snail cells were seeded in 6-well plates and serum starved for 24 h. and treated with PAM-9274 for 1 hr in the presence or absence of EGF stimulation (60 ng/ml for 5 min). RhoA and Rac1 activity was measured with G-LISA (colorimetric format, Cytoskeleton^{R}).

RESULTS



PAMs suppress spheroid formation. [Left] MiaPaCa-2-CSCs were grown in ultra-low adherent six well plates and in spheroid forming media DMEM F 12 with N2 and B2 supplement (Invitrogen) and exposed to increasing concentrations of PAMs (0-1000 nM) twice a week for two weeks. The spheroids were counted under a microscope and photographed. **[Right]** 10,000 CSCs per well were grown in regular DMEM media in 4 well chambered slides and exposed to PAMs (1000 nM) for additional 72 hrs. IF was performed using PAK4 antibody (1:250).



PAMs anti-tumor activity against PDAC CSCs. [A] Mice (n=6) harboring bilateral CSC tumors were exposed to 140 mg/kg KPT-9274 i.v. once a day for three weeks. Control mice (that reached ~1500 mg tumor size were exposed to KPT-9274 at day 32 daily for 1 week. **[B]** H&E staining of residual tumors from control group at day 32 and 49.

CONCLUSIONS

PAK4 plays a central role in PDAC stemness. PAK4 allosteric modulators (PAMs) reverse EMT and stemness in vitro.

PAM KPT-9274 shows remarkable anti-tumor activity in PDAC CSC derived xenograft

Lead PAM KPT-9274 has been introduced in Phase I (ClinicalTrials.gov: NCT02702492)