Inhibition of PAK4 attenuates renal cell carcinoma (RCC) growth

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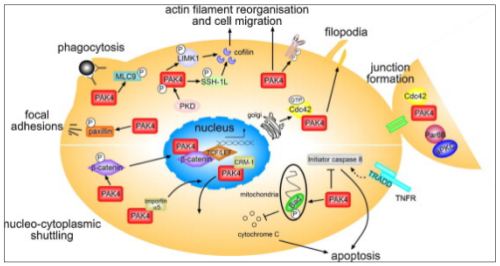
Introduction

- Renal cell carcinoma (RCC) is an increasingly prevalent cancer type that is frequently asymptomatic on presentation and is associated with poor responses and resistance even to the current targeted therapies.
- The p21-activated kinases (PAKs) are Rac1 and Cdc42 effectors that have generated significant interest as therapeutic targets in cancer.
- PAK4 is a mediator of filopodia formation and stabilizes β-catenin transcriptional activity and lies in a pathway integral to both nephrogenesis and cancer
- In most adult tissues, PAK4 is expressed at low levels, but overexpression of PAK4 is associated with uncontrolled proliferation, inappropriate cell survival, and oncogenic transformation.



Dart A, Wells C. "P21-activated kinase 4 – Not just one of the PAK
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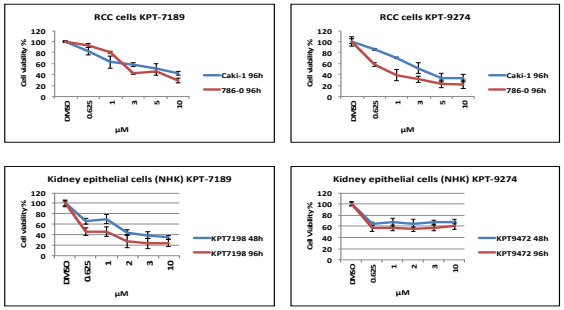
Procedure

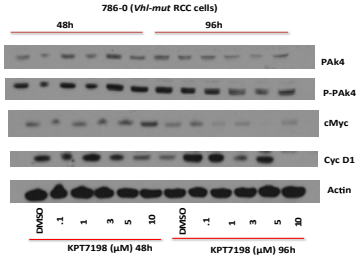
Materials:. Two human proximal tubule epithelial cancer cell lines,Caki-1 (vhl-wt) and 786-O (vhl-mut) were obtained from the American Type Culture Collection (Rockville, MD). Primary normal human kidney epithelial cell line, NHK, was from Lonza.
MTT assay: Cells were plated in 96 well plates, and after appropriate treatments, the cells were incubated in MTT solution/media mixture. Then, the MTT solution was removed and the blue crystalline precipitate in each well was dissolved in DMSO. Visible absorbance of each well at 540 nm was quantified using a microplate reader.
Immunoblotting: Immunoblotting was done according to a standard procedure using indicated antibodies.
Si RNA transfection: Caki-1 Cells were transfected either with scrambled oligos (cont si) or PAK4 siRNA from Invitrogen with a final concentration of 25 nM for 72h (n=3).
Xenograft mouse experiment: All animal procedures were performed in compliance with the University of California Institutional Animal Care and Use Committee. Male athymic Nu/Nu mice were injected with Caki-1 or 786-0 cells subcutaneously into the flank region. Tumor progression was monitored weekly with a caliper. When tumor sizes reached around 80-100 mm^3, mice were divided randomly into 4 groups (vehicle, Low dose of KPT-9274 (25 mg/kg), high dose (100 mg/kg) and sunitinib (40 mg/kg). All treatments were given orally.

Results

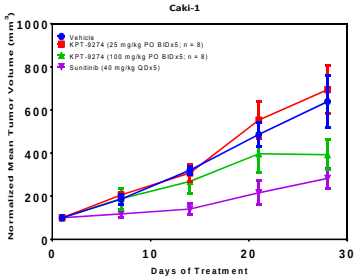
The PAK4 inhibitors (KPT-9274 and KPT-7189) dose-dependently inhibit cell viability in RCC cells but less so in the normal proximal epithelial primary cell lines NHK

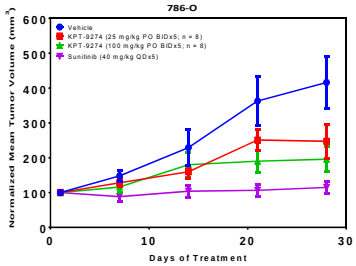


KPT-7189 downregulates PAK4 and downstream proteins in 786-0 RCC cells

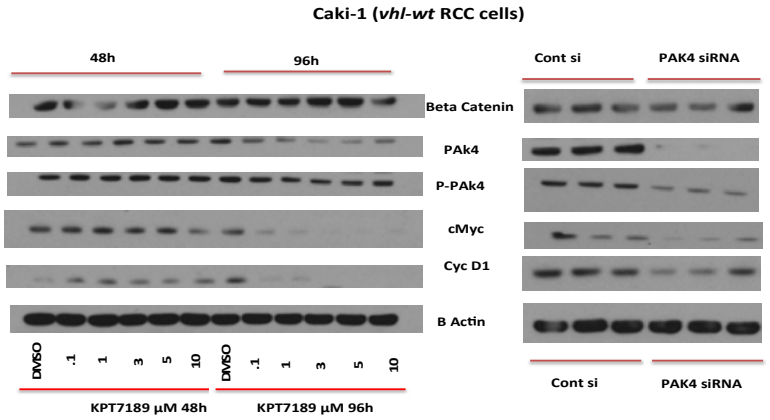


KPT-9274 inhibits RCC growth in two subcutaneous xenograft mouse models





Target protein analysis demonstrates specificity of KPT-7189 towards PAK4 in Caki-1 cells and suggests that its distal effects are mediated by C-Myc



Summary

- The PAK4 inhibitors KPT-9274 and KPT-7189 specifically target PAK4 protein, and concurrently decrease c-Myc and beta-catenin, in RCC cells.
- Cell viability is decreased on a dose-dependent manner in RCC cells and less so in normal kidney epithelial cells.
- KPT-9274 decreases growth of RCC in a subcutaneous xenograft model of this disease

Conclusion

- PAK4 attenuation by specific inhibitors is a novel therapeutic approach in RCC

Acknowledgement

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