NOVEL PAK4 INHIBITORS FOR PANCREATIC CANCER THERAPY

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INTRODUCTION

Background: Pancreatic cancer (PC) is a deadly disease in urgent need of novel molecularly targeted drugs. Gene copy number amplification studies in PC patient cohorts has shown amplification of the p21-activated kinase (PAK) family member PAK4. PAK4 acts as a key effector of the Rho family GTPases downstream of Ras signaling. Moreover, PAK4 protein is over-expressed in PC cell lines but not in normal human pancreatic ductal epithelial (HPDE) cells. Most importantly, RNA interference of PAK4 has been shown to suppress PC cell proliferation. These studies make PAK4 an attractive therapeutic target especially since direct targeting of Kras has been difficult. Nevertheless, the previously developed PAK4 Type I ATP competitive inhibitor (PF-3758309; tested in non-pancreatic models) was evaluated in a Phase 1 trial. It showed undesirable pharmacokinetic properties as well as no objective responses and has subsequently been discontinued. In order to fill this scientific void, we evaluated a new class of PAK4 allosteric modulators in pancreatic cancer models.

METHODS

We have identified a new class of PAK4 allosteric modulators that show anti-proliferative activity against several PC cell lines (IC50s <250nM) while sparing normal HPDE (IC50s 5 fold higher). Using multiple molecular biology techniques (growth inhibition assay, apoptosis, immunoblot, co-immunoprecipitation, small inhibitor (Si)-RNA, systems biology and fluorescence microscopy analyses), we tested the PAK4 allosteric modulator activity (in the presence and absence of -ve and +ve controls) in PC cells lines, PAK4 over-expressing Gemcitabine resistant (GEM-R) PC models and highly resistant flow sorted PC stem cells (CSC) that are triple positive for CD133+CD44+EpCam+ and undergo epithelial-to-mesenchymal transition (EMT). The toxicity and efficacy of these PAK4 modulators were evaluated in sub-cutaneous mouse models of PC.

RESULTS

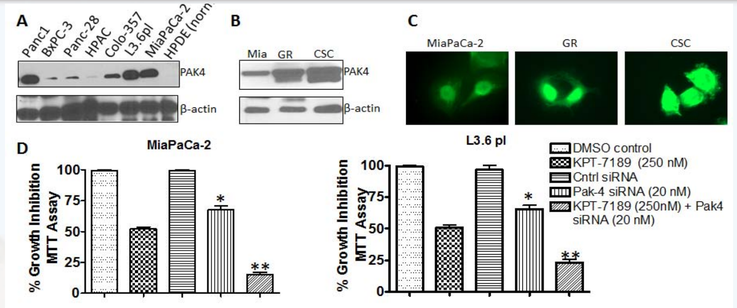

Figure 1. PAK4 as a potential therapeutic marker in PC. [A] Basal expression of PAK4 in a panel of PC cell lines & normal HPDE cells. PAK4 expression is directly correlated with PC resistance **[B]** Western blotting and **[C]** Immunofluorescence showing expression of PAK4 in MiaPaCa-2 (Mia), MiaPaCa-2 GEM resistant (GR) and MiaPaCa-2 derived (CSCs). **[D]** siRNA silencing of PAK4 suppresses PC cell growth * P<0.05 vs untreated and ** p<0.01 vs KPT-7189.

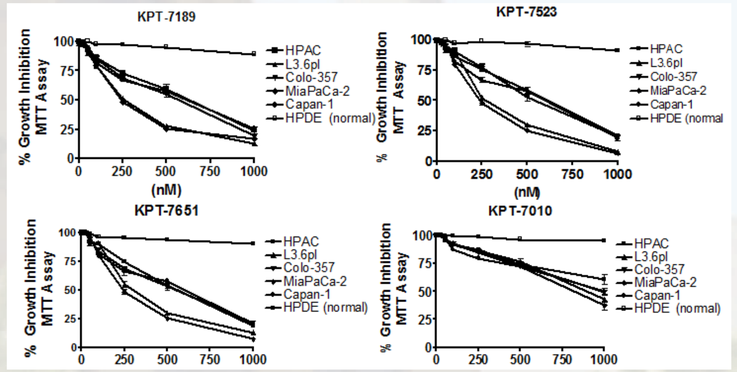

Figure 2. PAK4 inhibitors suppress proliferation of PC cells. MTT assay (72 hrs) showing anti-proliferative activity of analogs KPT-7189, KPT-7523, KPT-7651 and KPT-7010.

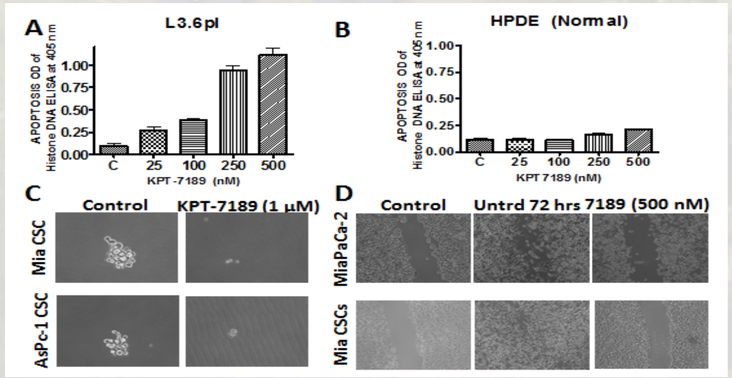

Figure 3. PAK4 inhibitors induce apoptosis [A & B] and suppress spheroid formation [C] and wound healing [D] in PC models.

RESULTS

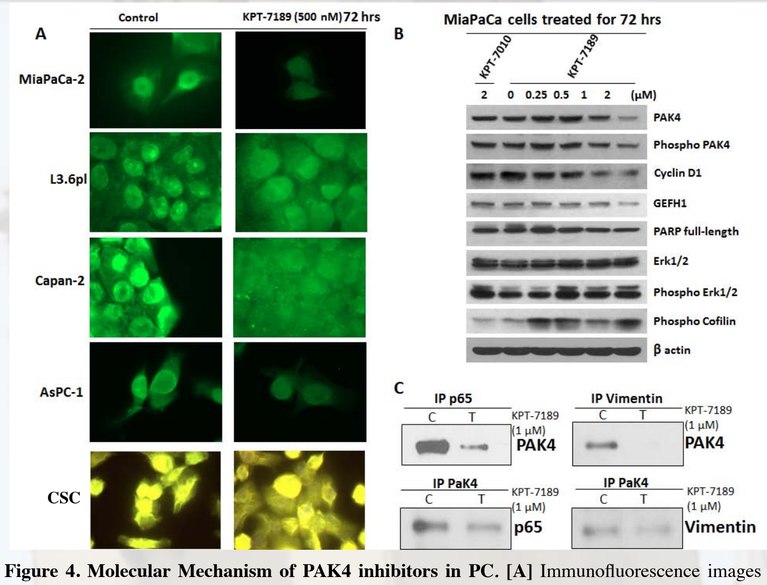

Figure 4. Molecular Mechanism of PAK4 inhibitors in PC. [A] Immunofluorescence images showing suppression of PAK4 protein expression in 4 PC cell lines and MiaPaCa-2 CSCs. **[B]** Western blot analysis showing suppression of PAK4 and downstream markers by KPT-7189 and not inactive analog KPT-7010. **[C]** Coimmunoprecipitation studies showing disruption of PAK4-p65 (NF-kB) and PAK4-vimentin interaction upon drug treatment in MiaPaCa-2 cells.

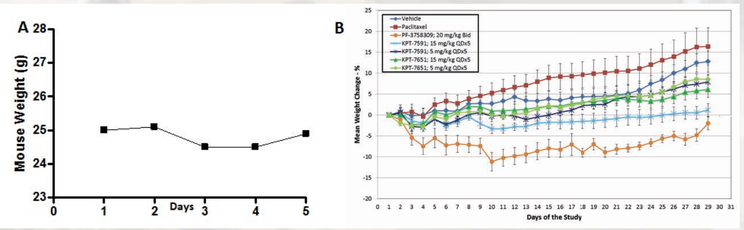

Figure 5. KPT-7651 administered orally is well tolerated by SCID mice. [A] Mice were administered KPT-7651 at 60 mg/kg. [B] Comparative body weight evaluations of different PAK4 allosteric modulator analogs to Pfizer compound. Sub-cutaneous efficacy studies are underway.

CONCLUSIONS

We have identified a new class of PAK4 allosteric modulators that show anti-proliferative activity against therapy resistant PC.

This is the first proof of concept study demonstrating PAK4i for the treatment of PC, warranting further clinical investigations .

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Background image: Old Main