Dual inhibition of NAMPT and PAK4 by KPT-9274 attenuates kidney cancer growth

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Introduction

- Renal cell carcinoma (RCC) is frequently metastatic at diagnosis and current treatments are often associated with resistance.
- PAK4/β-catenin signaling and NAD generation play key roles in survival, proliferation, and oncogenic transformation.
- PAK4 is a group II PAK isoform; PAK4 binds Cdc42 leading to modulation of nucleo-cytosolic trafficking of β-catenin.
- NAMPT inhibition results in significant depletion of NAD, a key cofactor in the TCA cycle, epigenetics (sirtuins), and DNA repair (PARP).

HYPOTHESIS

Dual inhibition of PAK4 and NAMPT causes apoptosis and cell cycle arrest which results in attenuation of RCC growth.

Materials and Methods

Cell lines: RCC cell lines, Caki-1 (vhl-wildtype), and 786-O (vhl-null) were from ATCC, and the "normal human proximal epithelial kidney cell line (RPTEC) was from Lonza. All cells were all maintained in DMEM media supplemented with 10% FBS, 100 units/mL streptomycin, and 100 mg/mL penicillin.

NAD+ and NADH measurement: Total NAD and NADH was assayed by luminescence measurement using The NAD/NADH-Glo™ Assay (Promega, Madison) following the manufacturer's protocol

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.

Cell cycle and apoptosis analysis: Both Cell cycle analysis and Annexin V & Dead Cell Assay was performed utilizing Muse™ Cell Analyzer from Millipore (Billerica, MA)

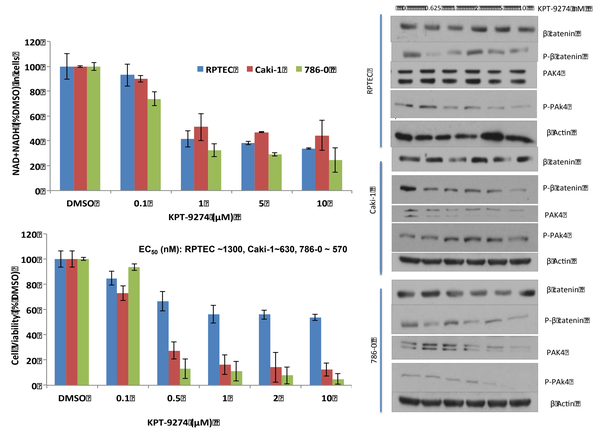
siRNA Transfection: Human PAK4 siRNA was from Thermo Fsher scentific. The transfection mixture was prepared in Opti-MEM GlutaMax medium from Invitrogen (Carlsbad, CA, USA) with siRNA and Lipofectamine RNAiMAX according to the manufacturer's protocol.

Enzymatic NAMPT Assay: Recombinant NAMPT activity was measured using a coupled-enzyme reaction system (CycLex NAMPT Colorimetric Assay Kit Cat# CY-1251: (CycLex Co., Ltd., Nagano, Japan)

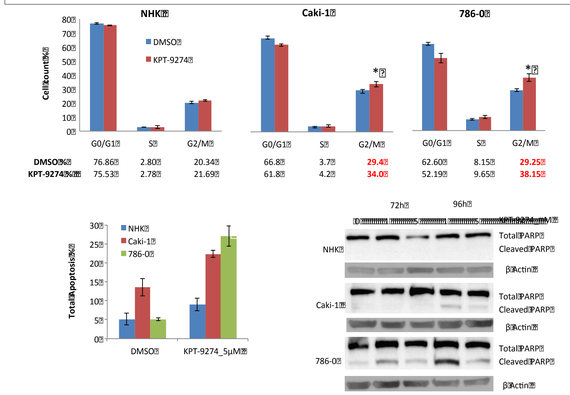
Xenograft mouse experiment: Male athymic Nu/Nu mice were injected with 786-O (human RCC) cells subcutaneously (DMEM:Matrigel 3:1) in the flank region. Tumor progression was monitored weekly by calipers using the formula: tumor volume in mm^3 = (length × width^2) /2.. KPT-9274 drug product (30% KPT-9274 API) or vehicle was administered by oral gavage twice daily for 5 days at 100 and 200 mg/kg. To determine any potential toxicity of the treatment(s), body weights of the animals were measured and signs of adverse reactions were monitored. On day 28 of treatment, the mice were euthanized and the tumor mass was determined. Tumor growth rate was calculated as (tumor volume on day X)/(tumor volume on day 1). Error bars indicates SEM.

Results

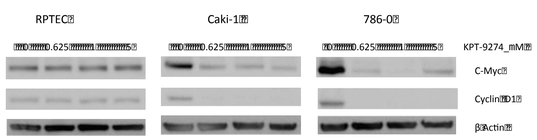
KPT-9274 depletes NAD, decreases cell viability (left), and attenuates the Wnt/β-catenin pathway (right) in RCC cells



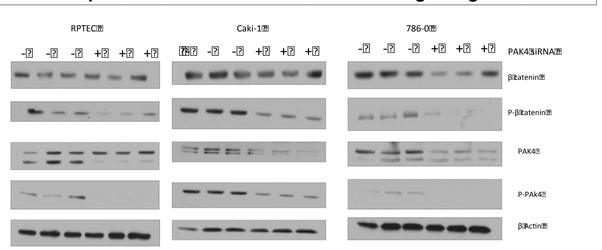
KPT-9274 causes G2/M arrest (top) and induces apoptosis (bottom) in RCC cells



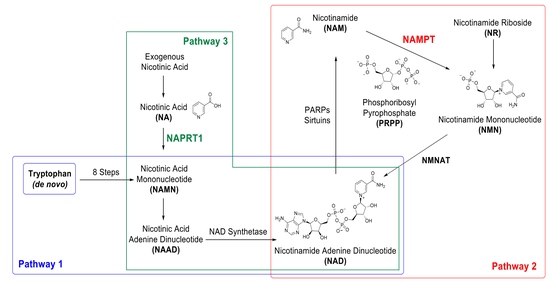
KPT-9274 down-regulates the Wnt/β-catenin targets cyclin D1 and c-Myc



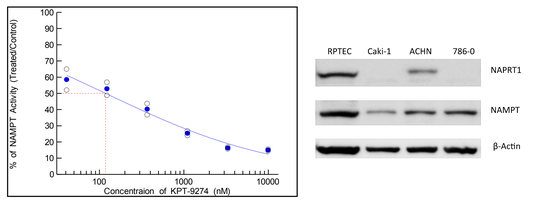
siRNA to PAK4 reproduces KPT-9274 effects on PAK4 signaling in RCC cells



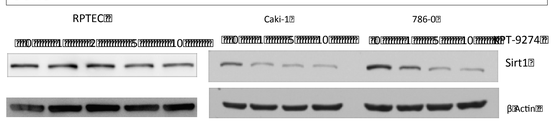
Schema for NAD biosynthesis pathways showing (1) de-novo and (2-3) the salvage pathways



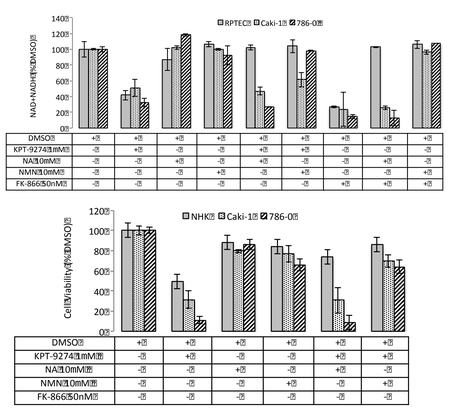
KPT-9274 attenuates NAMPT activity (left); basal NAPRT1 levels are decreased in RCC cells (right)



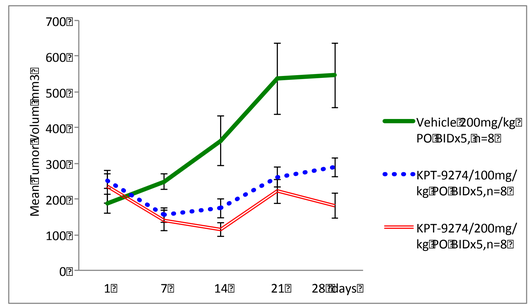
NAD-dependent Sirtuin-1 is attenuated by KPT-9274 in RCC cells

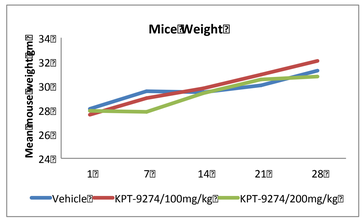


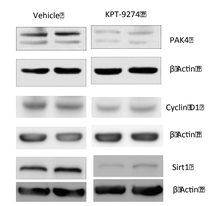
NAPRT1-deficit RCC cells do not rescue NAD levels with NA (top) and NAPRT1-deficit RCC cells do not rescue MTT by NA (bottom), emphasizing dependence of RCC on the NAMPT-dependent salvage Pathway 2.



KPT-9274 attenuates RCC growth in a mouse 786-O xenograft model, resulting in on-target effects with minimal mice weight loss







Summary

- A novel PAK4/NAMPT inhibitor decreases RCC tumor growth
- Both pathways (PAK4/β-catenin and NAD biosynthesis) appear to be active in RCC
- There was no evidence of toxicity in the mouse xenograft RCC model
- KPT-9274 is being evaluated in a phase 1 human clinical trial in solid tumors and lymphomas which will allow this data to be rapidly translated into the clinic for the treatment of RCC

Conclusion

KPT-9274 targets reprogrammed pathways in RCC and represents a novel small molecule approach for RCC treatment