Therapeutically targeting PAK4 as a Treatment for Breast Cancer

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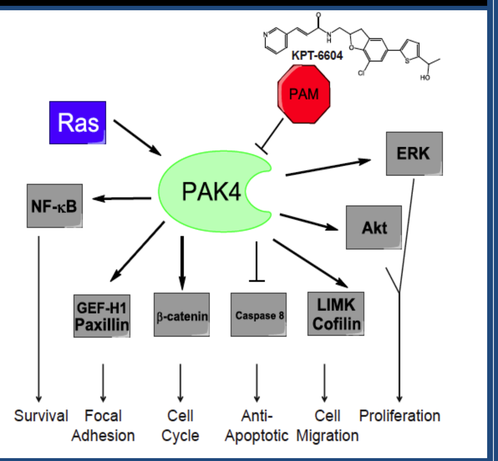
INTRODUCTION

The p21-activated kinase family of proteins promotes cell survival and plays an important role in cell proliferation, cell cycle regulation and cell shape determination. There are six mammalian PAK proteins which can be subdivided into two groups by sequence homology and mode of activation- Group A Paks consisting of Pak 1, 2 and 3 and Group B Paks consisting of Pak 4, 5 and 6. There is a growing list of evidence that PAK proteins are overexpressed in many cancer types, including colon, lung, and breast cancer. PAK4 is frequently overproduced in breast cancer, including Her2 positive and triple negative breast cancers, while it is expressed at low levels in normal mammary tissue. Our preliminary evidence showed that untransformed mouse mammary epithelial cells have low levels of PAK4 that become elevated when these cells are transformed by Her2 overexpression. Furthermore, PAK4 overexpressed in mammary epithelial cells leads to oncogenic transformation and tumorigenesis in mice. We hypothesize that PAK4 plays an important role in promoting mammary tumorigenesis, and that it can serve as an effective drug target for breast cancer treatment. To test this hypothesis, we have analyzed two orally available PAK4 Allosteric Modulators (PAM; KPT-8752 and KPT-9274), which reduce the steady state PAK4 protein level in cancer cells. We observed that when we treat breast cancer cells with KPT-8752, we severely block the cell growth. KPT-8752, is effective both in Her2 positive and triple negative breast cancer cells. Our results indicate that Pak4 is an important regulator of breast cancer cell growth. Experiments studying the effects of PAMs (KPT8752 and KPT9274) on tumorigenesis in animal models of breast cancer are ongoing.

METHODS

qPCR and Western Blot analysis were performed to measure Pak4 mRNA and protein levels, respectively. MTT assay was performed to monitor the effects of Pak4 inhibition on cell growth by incubating cells with PAMs at specified concentrations.

Flow cytometry analysis was performed to monitor the effects of Pak4 inhibition on apoptosis induction using Annexin V- Propidium Iodide staining.



Pak4 levels up regulated in multiple breast cancer cell lines

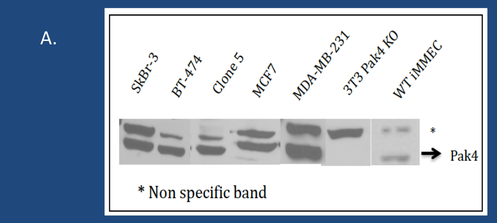


Fig.1 **Pak4 mRNA and protein expression levels in breast cancer cell lines**. Pak4 protein and mRNA levels were up regulated in, SkBr-3 (Her2+); BT-474 (PR+/Her2+) MCF7 (ER+/PR+)); MDA-MB-231 (Triple Negative) and iMMEC overexpressing Her2 (Clone 5) as compared to WT iMMECs, as analyzed by a. Western Blot and b. qPCR

Pak4 Allosteric Modulators block Pak4 protein levels and Pak4 associated signaling pathway

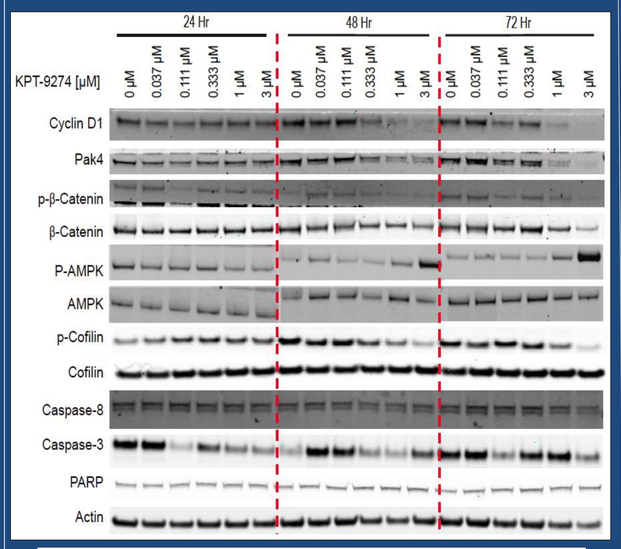


Fig.2 **PAMs inhibit Pak4 signaling in MDA-MB-231.** MDA-MB-231 cells were treated with varying amounts of KPT9274 for 24, 48 and 72hrs Treatment of cells with KPT9274 for 48 and 72hrs showed decrease in Pak4 protein and signaling along with key downstream effectors of cell cycle (B-catenin, Cyclin D1); cell migration (cofilin) and Autophagy (AMPK)

Pak4 Allosteric Modulators induce transcriptional changes in TNBC

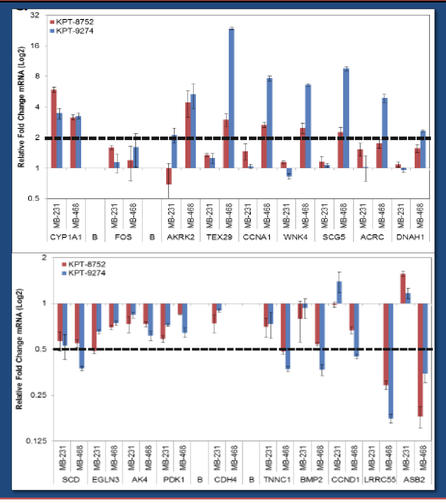


Fig3. **PAMs change the transcriptional network in TNBC cells**. MDA-MB-231 and MDA-MB-468 were treated with DMSO or 1μm KPT8752 for 24 and 48hrs. Total RNA was collected and used for deep sequencing (Selah Genomics). RT-PCR Analysis of a. Up regulated and b. Down-regulated genes in both TNBC cells.

Pak4 Allosteric Modulators block cell growth in multiple breast cancer cell lines

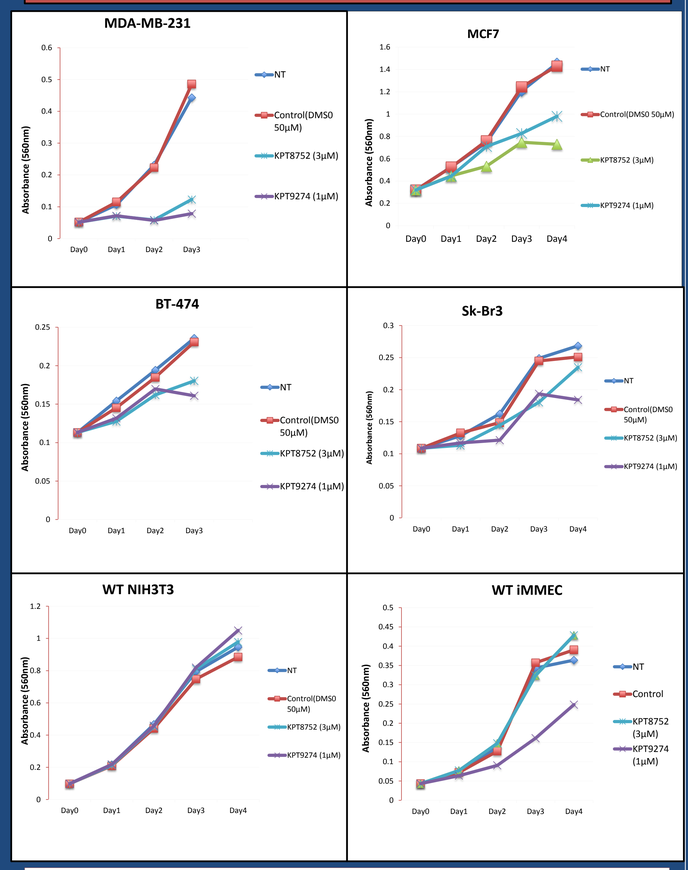


Fig. 4 **Effect of Pak4 inhibition on breast cancer cell growth.** Effect of Pak4 inhibition on cell growth was analyzed by MTT assay**.** Breast cancer cells expressing high levels of Pak4 were plated in tissue culture plates, treated with PAMs at the specified concentrations, and absorbance was measured at 560nm which corresponds to the number of viable cells. Treatment with PAMs blocked cell growth of MDA-MB-231; MCF7 and BT-474; with minimalistic effects on SkBr-3.

Pak4 Allosteric Modulators induce apoptosis in breast cancer cells

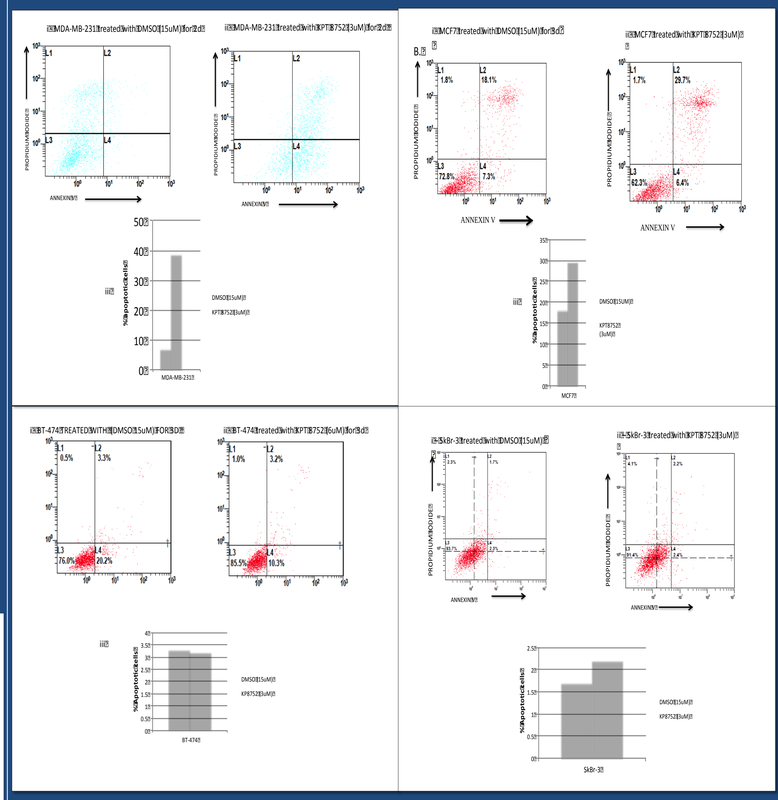


Fig.**5 PAMs induce apoptosis in breast cancer cells**. Breast cancer cells expressing high levels of Pak4 were incubated with DMSO (15uM) or KPT8752 (3uM) for 72hrs. These cells were then stained with Annexin-V and Propidium Iodide to analyze effect of Pak4 inhibition on cell apoptosis. Treatment with KPT8752 induced apoptosis in MDA-MB-231 and MCF7, while SkBr-3 and BT-474 cells were unaffected.

CONCLUSIONS

- Pak4 plays an important role in cell proliferation, cell survival and cell cycle regulation.
- Pak4 Allosteric Modulators (PAMs) decrease Pak4 protein levels and block Pak4 associated signaling pathways.
- PAMs block cell growth of breast cancer cells, while sparing normal cells.
- Her2 positive breast cancer cells appear to be resistant to PAMs.

Future Studies-

- Analyze the effects of Pak4 inhibition on cell migration.
- Analyze the effects of Pak4 inhibition on mammary acinar structure formation.
- Mice xenograft studies in which breast cancer cells implanted onto mammary fat pads of mice treated with PAMs; the rate, incidence and size of tumors will be subsequently observed.

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