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Subcellular localization of p21-Activated Kinase 6 (PAK6)

Ciny John and Michael L. Lu

Department of Biomedical Science, Charles E. Schmidt College of Medicine, Florida Atlantic University, Boca Raton, FL

The protein p21-activated Kinase 6 (PAK6) belongs to the PAK family of serine/threonine protein kinases known to play important roles in various cellular processes and it has been found to be overexpressed in primary and metastatic prostate cancer. We believe that investigating the subcellular localization of PAK6 will provide insight into normal physiological and morphological functions of the kinase and augment our understanding of its role in cancer. In current study, we determined the subcellular localization of PAK6 using immunofluorescence and of green fluorescence protein- (GFP) tagged PAK6 by confocal microscopy. At resting stage, PAK6 localizes primarily at plasma membrane. Upon activation by forskolin, a protein kinase A (PKA) activator, PAK6 translocates from the plasma membrane to cytosol in LAPC4 and PC3 prostate cancer cells. In addition, double immunofluorescence staining of MCF7 breast cancer cells revealed that PAK6 co-localizes with E-cadherin at adherens junction. These results suggest that PAK6 may play a role in signal transduction at adherens junction. Using the BiFC (Bimolecular fluorescence complementation) technique, it is demonstrated that PAK6 exists as homodimers at the plasma membrane. Consistent with activation-induced PAK6 translocation, activation of PAK6 by forskolin causes the PAK6 dimers to translocate to the cytoplasm. PAK6 also localizes on the membrane of the extending neuronal processes, suggesting the possible role of PAK6 in outgrowth of neuronal processes. These results suggest that PAK6 may have multiple functions, including adherens junction signaling and neuronal process formation.

Subcellular Localization of p21- Activated Kinase 6 (PAK6)

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Abstract

Using Confocal microscopy, we have studied the subcellular localization of PAK6 using immunofluorescence and of green fluorescence protein- (GFP) tagged PAK6. At resting stage, PAK6 localizes primarily at the plasma membrane. Upon activation by forskolin, a protein kinase A (PKA) activator, PAK6 translocates from the plasma membrane to cytosol in LAPC4 and PC3 prostate cancer cells. In addition, double immunofluorescence staining of MCF7 breast cancer cells revealed that PAK6 co-localizes with E-cadherin at adherens junction. These results suggest that PAK6 may play a role in signal transduction at adherens junction. Using the BiFC (Bimolecular fluorescence complementation) technique, it is demonstrated that PAK6 exists as homodimers at the plasma membrane. Consistent with activation-induced PAK6 translocation, activation of PAK6 by forskolin causes the PAK6 dimers to translocate to the cytoplasm. PAK6 also localizes on the membrane of the extending neuronal processes, suggesting the possible role of PAK6 in outgrowth of neuronal processes. These results suggest that PAK6 may have multiple functions, including adherens junction signaling and neuronal process formation.

Introduction

p21-activated Kinase 6 (PAK6) belongs to the PAK family of serine/threonine protein kinases known to play important roles in various cellular processes, including cytoskeletal rearrangement, cell survival, apoptosis, and signaling in the MAP kinase pathway[2]. PAK6 was first identified as an Androgen Receptor (AR) interacting protein cloned in prostate cancer cells, and it was later found to be overexpressed in primary and metastatic prostate cancer cell lines[1]. Cytosolic PAK6 has been previously shown to translocate with AR to the nucleus where it inhibits its transcriptional activity in prostate cancer cells[3]. More recently, PAK6 has been found to localize to the plasma membrane.

Methods

Investigating the subcellular localization of PAK6 will provide insight into normal physiological and morphological functions of PAK6 and augment our understanding of its role in cancer.

Confocal Microscopy was used to image:

- Immunofluorescence staining of PAK6 using the anti-PAK6 antibody, 34B9
- Expression of green fluorescence protein- (GFP) tagged PAK6
- Bimolecular fluorescence complementation (BiFC) of PAK6

Results

Double Immunofluorescence staining of MCF7 breast cancer cells for exogenously expressed PAK6 and PAK6 mutant localization

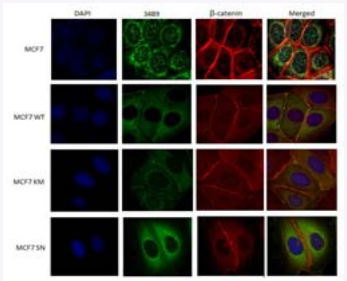

Figure 1: MCF7 and MCF7 cell lines exogenously expressing wild type (WT), kinase dead (KM) and kinase constitutively active (SN) PAK6 were stained with 34B9, an anti-PAK6 antibody, and co-stained with anti-β-catenin. β-catenin interacts with the cytoplasmic domain of E-cadherin, an adhesion molecule, at adherens junction in cell-to-cell contact. PAK6 co-localizes with β-catenin, suggesting that PAK6 localizes to the membrane and may play a role in signal transduction at the adherens junctions.

Double Immunofluorescence staining of LAPC4 prostate cancer cells for endogenously expressed PAK6 localization

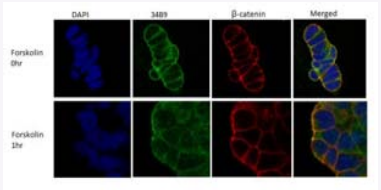

Figure 2: LAPC4 cells endogenously overexpressing PAK6 were stained with 34B9 and co-stained with anti-β-catenin. PAK6 again shows colocalization with β-catenin on the plasma membrane, at the resting state. The cells were treated with forskolin, to promote activation, for 1 hour. After forskolin treatment, the activated PAK6 translocates from the membrane and into the cytoplasm.

Results

Confocal imaging of N2A cells exogenously expressing GFP tagged PAK6

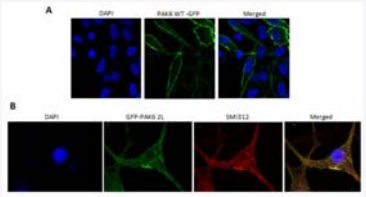

Figure 3: A) N2A cells were transfected with GFP tagged wild type PAK6 to image its localization in neuroblastoma cells. PAK6 appears to localize to the membrane as seen in other cell types. B) N2A cells were transfected with a mutant form of PAK6 in which binding to the membrane is inhibited (2L). Imaging of these cells show cytosolic localization of PAK6 as would be expected.

Double immune-fluorescence staining for localization of endogenously expressed PAK6 in primary cortical neuronal cells

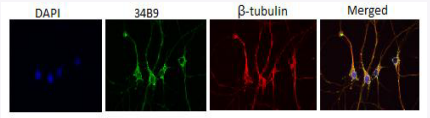

Figure 4: Since PAK6 is predominantly found in the brain, primary cortical cells were stained with anti-PAK6 antibodies, and co-stained with anti-β-tubulin which stains microtubules in neuronal cells. PAK6 can be found on the extending processes of the neuronal cells indicating a possible role in processes formation.

Conclusion

- *PAK6 localizes to the membrane at cell-to-cell contact regions in MCF7 cells and may be involved in signal transduction at the adherens junctions*
- *Both endogenously expressed PAK6 in LAPC4 cells and exogenously expressed wild type PAK6 in N2A cells localize on the plasma membrane.*
- *PAK6 localizes on the membrane of neuronal cells and may play a role in the formation of neuronal processes*
- *PAK6 forms homodimers on the membrane at resting state and translocates to the cytoplasm once activated by Forskolin. This may be a transient activation, and upon inactivation after prolonged exposure, it translocates back to the membrane.*

Results

Bimolecular Fluorescence Complementation Assay

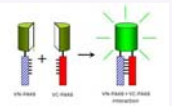

Figure 5: The basic principle of BiFC in which the interaction between two proteins is analyzed by the association between the two fluorescent protein fragments brought together by the interaction[4]. VN-PAK6 and VC-PAK6 were co-transfected in HeLa cells to image PAK6 interaction and localization.

BiFC of complementary GFP tagged PAK6 for localization after forskolin stimulation over time in HeLa cells

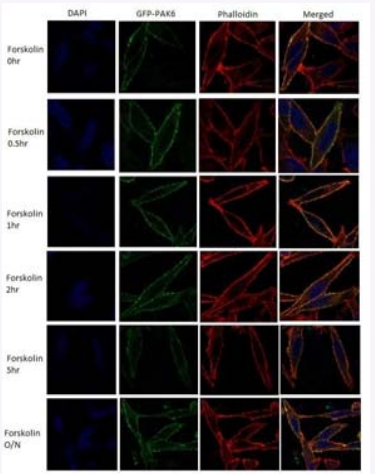

Figure 6: HeLa cells were cotransfected with VN GFP-PAK6 and VC GFP –PAK6 and imaged after forskolin treatment to promote activation for 30 minutes, 1hour, 2 hours, 5hours, and overnight. Cells were stained with Phalloidin for cytoskeletal staining. Untreated cells and cells treated for 30 minutes, 1hour and 2 hours showed localization of PAK6 to remain on the membrane. After 5 hours, PAK6 begins to translocate into the cytoplasm. However, after overnight treatment, PAK6 localizes back on the membrane. The BiFC technique revealed the PAK6 forms homodimers on the membrane. This further confirms that PAK6 localizes on the membrane at resting state, and translocates to the cytoplasm when activated.

References

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