World Academy of Science, Engineering and Technology
International Journal of Bioengineering and Life Sciences
Vol:17, No:09, 2023

Trans-Activator of Transcription-Tagged Active AKT1 Variants for Delivery to Mammalian Cells

Authors : Tarana Siddika, Ilka U. Heinemann, Patrick O'Donoghue

Abstract : Protein kinase B (AKT1) is a serine/threonine kinase and central transducer of cell survival pathways. Typical approaches to study AKT1 biology in cells rely on growth factor or insulin stimulation that activates AKT1 via phosphorylation at two key regulatory sites (Threonine308, Serine473), yet cell stimulation also activates many other kinases and fails to differentiate the effect of the two main activating sites of AKT1 on downstream substrate phosphorylation and cell growth. While both AKT1 activating sites are associated with disease and used as clinical markers, in some cancers, high levels of Threonine308 phosphorylation are associated with poor prognosis while in others poor survival correlates with high Serine473 levels. To produce cells with specific AKT1 activity, a system was developed to deliver active AKT1 to human cells. AKT1 phospho-variants were produced from Escherichia coli with programmed phosphorylation by genetic code expansion. Tagging of AKT1 with an N-terminal cell penetrating peptide tag derived from the human immunodeficiency virus trans-activator of transcription (TAT) helped to enter AKT1 proteins in mammalian cells. The TAT-tag did not alter AKT1 kinase activity and was necessary and sufficient to rapidly deliver AKT1 protein variants that persisted in human cells for 24 h without the need to use transfection reagents. TAT-pAKT1T308, TAT-pAKT1S473 and TAT-pAKT1T308S473 proteins induced selective phosphorylation of the known AKT1 substrate GSK-3αβ, and downstream stimulation of the AKT1 pathway as evidenced by phosphorylation of ribosomal protein S6 at Serine240/244 in transfected cells. Increase in cell growth and proliferation was observed due to the transfection of different phosphorylated AKT1 protein variants compared to cells with TAT-AKT1 protein. The data demonstrate efficient delivery of AKT1 with programmed phosphorylation to human cells, thus establishing a cell-based model system to investigate signaling that is dependent on specific AKT1 activity and phosphorylation.

Keywords : cell penetrating peptide, cell signaling, protein kinase b (AKT1), phosphorylation
Conference Title : ICMBB 2023 : International Conference on Molecular Biology and Biochemistry
Conference Location : Toronto, Canada
Conference Dates : September 18-19, 2023