

PAK1 inhibitor alters the recruitment of downstream effector proteins including the ESCRT-1 machinery

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Cytokinesis is the final step of cell division which divides the cytoplasmic contents of a parental cell into two daughter cells. The process of cytokinesis in animal cells is initiated after chromatid separation during the anaphase when a contractile ring is formed. This ring assembles equatorially, at the cell cortex, initiating the formation of a cleavage furrow, followed by subsequent primary and secondary ingression eventually forming a mid-body between the two cells. The process of cytokinesis is coordinated by the sequential recruitment of several proteins downstream of active RhoA, at the mid-body which trigger further localization of actin filaments. p21 activated kinases act downstream of Rho GTPases and are involved in essential cytoskeletal dynamics. Polo-like kinases (PLK) act downstream of PAK, and its kinase activity is regulated by PAK1. Inhibition of PAK activity formed abnormal spindle fibres due to disoriented regulation of PLK. Later during cytokinesis, a complex called the Endosomal Sorting Complex (ESCRT) is recruited at the mid-body which is involved in scission and membrane remodelling. Abscission is the final step of cytokinesis which terminates with the final cleavage of the mid-body equally into two. PAK1 kinase activity is strongly inhibited by IPA 3. Integrins are an important family of cell surface receptors found in all metazoans which promote anchorage. Lack of anchorage in non-transformed cells leads to the failure of cytokinesis. A non-transformed cell takes 3.5-4 hours to complete the process of cytokinesis. Hence in our study we tried to look if the inhibition in PAK kinase activity caused by IPA-3 led to inhibition of the recruitment of the downstream effector proteins like phosphorylated PAK, PLK, and also the ESCRT-1 machinery. We found that IPA-3 apart from delaying the normal duration of cytokinesis to 7 hours, also showed strong inhibitory effects on the recruitment of TSG101, phosphorylated PAK, and PLK when it was incubated with the cells for longer time-periods. This effect disappeared when IPA-3 was incubated with the cells for shorter spans of time. Finally we were able to deduce that not only the proteins acting downstream of active RhoA are interrelated to each other with each contributing to the process of cytokinesis in its own specified time, but also the inhibition of a preliminary player PAK indicated similar inhibitory effects on the downstream effector proteins. Further understanding of this process can aid the design of treatment of tumors which exhibit the potential of undergoing anchorage independent growth (AIG).

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