The interactome of human Nek7 differs from that of Nek6 and substrate recognition is mediated by the N- and C-terminal domains

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Nek7 is a centrosomal NIMA-related kinase (Nek) required for progression of the cell cycle and its deregulation has been correlated with uncontrolled cell proliferation and tumor progression. Nek6 and Nek7 consist of a short non-conserved and disordered N-terminal regulatory domain and a conserved C-terminal catalytic domain, which represent about 86% identity. It has been shown that Nek6 and Nek7 have independent roles in mitotic spindle formation and cytokinesis. However, the Nek7 interacting protein partners and, hence, Nek6 and Nek7 independent signaling pathways in cell remained so far elusive. To provide new insights for Nek7 function and to further elucidate the molecular basis for the functional independency of Nek6 and Nek7 we carried out a yeast two-hybrid screen for Nek7 and identified 25 interacting partners belonging to 12 functional categories. From Nek6 and Nek7 chimeric constructs we performed a comparative yeast two-hybrid assay in 3-AT a gradient using selected Nek6 and Nek7 respective interacting partners. The results revealed that both the N- and C-terminal of Nek6 and Nek7 are involved in mediating the interactions with the different partners. Now, in vitro tests are being performed to test the ability of Nek6 and Nek7 N/C-terminal domains in substrate recognition and phosphorylation. To test if Nek7 interacting partners are also its substrates we expressed hNek7 in E. coli and purified it by affinity chromatography. Recombinant Nek7 phosphorylated in vitro its interaction partners tubulin beta-2B chain (TUBB2B) and coiled-coil and C2 domain-containing protein 1A (CC2D1A). Furthermore, we labeled endogenous hNek7 and α-tubulin and observed that hNek7 co-localizes with α-tubulin in the cytoplasm in a perinuclear region reinforcing the participation of Nek7 in the regulation of the microtubule and centrosome.

Keywords: cell cycle, Nek7, cancer, yeast two-hybrid, phosphorylation

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