ABSTRACT

Optineurin (OPTN) is a versatile cytosolic adaptor protein mainly involved in selective autophagy, vesicular trafficking, inflammatory signalling, and maintaining cellular homeostasis. Mutations and dysregulation of OPTN are strongly linked to several neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS) and primary open-angle glaucoma (POAG), prompting extensive studies of its cellular roles. Although OPTN has been observed in various intracellular compartments, such as mitochondria, the Golgi apparatus, endosomes, and the nucleus, its exact function during cell division remains unclear. Previous research has shown mitotic defects and delayed metaphase-to-anaphase transition in OPTN-depleted cells, leading us to examine the localization and movement of OPTN during different cell cycle stages. Using immunofluorescence in several human cell lines, including HeLa, HEK293, and SH-SY5Y, along with wide-field, confocal, and structured illumination microscopy (SIM), we found that OPTN consistently localizes to the centrosome. It remains associated with duplicated centrioles and mitotic spindle structures during division and is also observed at the daughter cell junctions during cytokinesis. Interestingly, at the G1/S transition, OPTN exhibited a preferential, asymmetric distribution between the two centrioles, suggesting a possible association with centriole maturation or functional asymmetry during cell cycle progression. We identified these sites of OPTN localization utilizing centrosomal and mitotic markers such as Aurora A kinase, pericentrin, pericentriolar material 1 (PCM1), centrosomal protein of 170 kDa (Cep170), γ-tubulin, and β-tubulin. This allowed us to designate OPTN as a previously unrecognized component of cell division assemblies. To further understand the spatio-temporal dynamics, we performed experiments on synchronized HeLa cells and conducted microtubule disruption assays. These revealed that OPTN gradually detaches from centrosomes during mitosis and relocates along spindle-associated cytokinetic structures, accumulating at the spindle midzone and eventually at the midbody. After cytokinesis, OPTN re-emerges at centrosomes during the G1 phase alongside the maturation of the PCM. Microtubule disruption and regrowth assays indicated that OPTN's departure from the PCM depends on microtubules, but its recruitment does not. Overall, this research uncovers the dynamic trafficking of OPTN linked to the centrosome during cell division and offers new insights into its possible role in regulating cell cycle progression.