

SUPER-RESOLUTION IMAGING FOR ARL13B AND ACETYLATED TUBULIN DISTRIBUTION IN THE PRIMARY CILIA

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ABSTRACT

The primary cilium is a solitary, non-motile organelle emerging from the cell surface of most mammalian cell types during quiescent status and serves as a mechanical sensor [1]. It consists of one mother centriole, the ciliary membrane and the 9+0 ciliary axoneme [2]. The size of the primary cilium is less than 10 μm long and 2 μm in diameter. Immunofluorescence super-resolution microscopy is applied for studying ciliary markers, acetylated tubulin (Acet-T) and Arl13b in human embryonic kidney 293 and adenocarcinoma epithelial A549. Super-resolution imaging systems including STED, Airyscan and DeltaVision OMX SR are used for analysis. Arl13b and Acet-T in the primary cilia on the non-mitotic cells are found co-localized under widefield and confocal imaging. STED imaging shows the intercalating pattern of Arl13b and Acet-T from the middle section to the tip of the cilia in 293 cells. For A549 cells, Arl13b accumulates prominently at the base and tip positions. The primary cilia disassemble during mitosis, in which Acet-T and Arl13B seem to relocate to the cytoplasm, in which Acet-T is arranged into bundle-like structure. More signals of Arl13b are detected gathering around the reappeared nuclei, and the bundle-like Acet-T appears in pairs at the opposite direction or in the form of three pointed stars in the neighboring cells. This study has shown that mitotic microtubules are enriched in Acet-T both in normal and cancer cells as described in HeLa cells [3]. The 293 cells have shown that Arl13b and Acet-T are aligned on both sides inside the organelle with super-resolution of OMX. STED further provides the evidence that Arl13b and Acet-T intercalate inside the space of the middle part of the primary cilia instead of overlapping.

REFERENCES

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