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CSPP-L and EB3 localize to centriolar satellites and are required for satellite-dependent recruitment of ciliopathy proteins to the centrosome

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Objective

Centrosome/Spindle Pole associated Protein 1 (*CSPP1, JBTS21*) mutations cause Joubert syndrome (JBTS) and JBTS-related ciliopathies. The large protein isoform CSPP-L is a ciliary protein required for ciliogenesis and stabilization of the ciliopathy protein RPGRIP1L (NPHP8/JBTS7/MKS5/FTM) at the ciliary transition zone (TZ). However, RPGRIP1L is dispensable for ciliogenesis and the mechanism by which CSPP-L promotes ciliogenesis is unclear.

Methods

We applied immunogold electron, immunofluorescence and fluorescence live cell microscopy to determine localization of CSPP-L at high spatial and temporal resolution. We elucidated the functional interplay of CSPP-L with centriolar satellites in hTERT-RPE1 and HeLa cells using biochemical analysis of CSPP-L complexes, siRNA modulated gene expression and quantitative immunofluoresecence microscopy.

Results

We show that CSPP-L localizes to centriolar satellites, in addition to axonemal microtubule (MT) plus ends and the TZ, and that the MT plus end-tracking protein EB3 also localizes to satellites. CSPP-L complexed with the known satellite component PCM1 and GFP-CSPP-L showed satellite-like dynamics. Importantly, CSPP-L depletion decreased formation of PCM1, CEP290 and EB3-comprising satellites, whereas depletion or inactivation of EB3 impaired centrosomal localization of CSPP-L.

Conclusion

Our results identify a new link between MT plus ends and centriolar satellites, and suggest that CSPP-L contributes to ciliogenesis by promoting EB3- and dynein-dependent recruitment of satellite components to the centrosome.

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