博士論文の要約

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論文題目　Functional analysis of Cep57 in centrosome biogenesis

Centrosomes are non-membrane-bound organelles that serve as the major microtubule-organizing centers (MTOCs) in most animal cells and participate in diverse biological processes. A centrosome consists of a pair of centrioles and surrounding pericentriolar material (PCM). Centrosome abnormalities have been linked to human diseases such as cancer and ciliopathies. Centrosome duplication occurs once per cell cycle, which is essential to ensure the correct number of centrioles. Toward the G1-to-S transition, centriole formation begins with the assembly of the cartwheel structure that mainly dictates the universal radial nine-fold symmetry of centrioles, followed by attachment of peripheral centriolar microtubules. During mitosis, the centrosomes act as MTOCs to ensure the robust formation of mitotic bipolar spindle and proper chromosome segregation. At this stage, surrounding PCM drastically expands and acquires MTOC activity. Each newly-formed daughter centrioles are orthogonally connected to each mother centriole until late mitosis. The loss of connection between the mother and daughter centrioles occurs after cytokinesis with the disassembly of expanded PCM. The disconnection process is called “centriole disengagement” and thought to be a licensing step for centriole duplication in the next cell cycle. Therefore, the timing of centriole disengagement must be tightly regulated. However, the mechanism underlying centriole engagement remains elusive.

Although it is reported that Cep57 is responsible for mosaic variegated aneuploidy (MVA) syndrome, the role of Cep57 in centrosome biogenesis is poorly understood. In this study, I addressed the role of Cep57 in centriole engagement and

PCM organization and showed that Cep57 is essential for PCM organization and centriole engagement during mitosis. I revealed that Cep57 depletion results in precocious centriole disengagement and PCM disorganization in mitosis. Interestingly, such precociously-disengaged daughter centrioles aberrantly recruit PCM components and acquire ectopic MTOC activity, which thereby appears to cause chromosome segregation errors and aneuploidy in human cells. Moreover, my study for the first time provides evidence that precociously-disengaged centrioles can be unequally distributed into two daughter cells, which also leads to chromosome segregation errors and aneuploidy. Given these results, I also tested whether my finding in Cep57-depleted human culture cells is also true in MVA patients' cells. As expected, MVA patients' cells also exhibit similar defects, such as precocious centriole disengagement and PCM disorganization, suggesting a potential cause of the MVA disease.

To understand how Cep57 contributes to PCM organization and centriole engagement, I examined the precise localization pattern of Cep57 using super-resolution (STED) microscopy. STED analysis show that Cep57 localizes at the proximal ends of mother centrioles. I next searched for a PCM component directly associated with Cep57 and found that Cep57 directly binds to the well-conserved PACT domain of PCNT. The PACT domain has been utilized for targeting a protein of interest to centrioles in the fields since it was discovered. However, the exact binding partner of the PACT domain has not yet been determined. My study shows that Cep57 is the in vivo anchor for the PACT domain. Moreover, numerous mutations in *PCNT* gene have been reported to cause mosaic variegated primordial dwarfism type 2 (MOPD2). Among them, the p.K3154del mutation seems to be unique as it is pathogenic as a result of missing only a single amino acid within the PACT domain. I then tested whether the mutations within the PACT domain affect the Cep57-PCNT interaction and found that the MOPD2-related *PCNT* mutation impairs the interaction and phenocopies the mitotic phenotypes

provoked by depletion of endogenous Cep57, suggesting a potential cause of the MOPD2 disease. Finally, I show that Cep57 is evolutionarily conserved across species. Using BLAST search, I succeeded in identifying a 36-amino acid region of homology in other species. Accordingly, I term the conserved short region ‘PINC (present in the N-terminus of Cep57) motif’. The PINC motif of Cep57 is involved in its centriolar localization and the PCNT binding.

In conclusion, these findings lead me to propose that the conserved Cep57-PCNT module provides a critical interphase between the centriole core and PCM and is critical for centriole engagement and PCM organization during mitosis.