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題目(和文)	CLIP-170は細胞接着面におけるダイニン配置を制御することにより T細胞活性化における中心体の細胞接着面近傍への移動に必須の役割を果たしている
Title(English)	CLIP-170 is essential for MTOC repositioning during T cell activation by regulating dynein localisation on the cell surface
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（Summary）

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Introduction: T cell activation is an essential step of the immune response. It is initiated by the recognition of the specific antigen displayed on the surface of an antigen-presenting cell (APC). This activation triggers the immune response in T cells, including cytokine production such as interleukin 2 (IL-2), and the dynamic reorganization of actin and microtubule cytoskeletons. At almost the same time, microtubule-organizing centre (MTOC), or centrosome in this case, undergoes dynamic repositioning and is moved to the immunological synapse, where secretory vesicles are accumulated to allow focused secretion against the target cell. For example, an inhibitory receptor CTLA-4, which is used as a target of cancer immunotherapy, is regulated by MTOC repositioning. In contrast to the importance of MTOC repositioning during T cell activation, the molecular mechanisms underlying this process remain unknown.

As for the driving motive force of MTOC movements, several lines of evidence have shown the involvement of cytoplasmic dynein, the major microtubule minus-end-directed motor protein, in MTOC repositioning. As one of two mechanisms for targeting dynein to the plus, a subset of plus-end tracking proteins (+TIPs), such as +TIP end-binding protein EB1, CLIP-170 (cytoplasmic linker protein 170) and dynactin, is known to recruits dynein to the plus-end. CLIP-170, the key molecule in targeting dynein to the plus end, is also responsible for regulating microtubule dynamics. CLIP-170 phosphorylated by AMPK (AMP-activated protein kinase) rapidly dissociated from the microtubule and promotes efficient microtubule polymerization. How dynein is translocated to the immunological synapse in T cells remain uncovered. In this study, to elucidate this mechanism, I focused on the role of CLIP-170 in MTOC repositioning and the interaction with dynein involved in T cell activation.

Results:

1. CLIP-170 regulates MTOC repositioning and T cell activation

First, CLIP-170 knockdown experiments were carried out to assess the functional contribution of CLIP-170 to MTOC repositioning. The CLIP-170 knockdown cells stimulated with coated anti-CD3ε/anti-CD28 antibodies showed disturbed microtubule frameworks and decentred the MTOC position. The relative IL-2 expression in CLIP-170 knockdown cells decreased significantly compared to wild-type cells. Taken together, CLIP-170 knockdown impaired MTOC repositioning and full activation of T cells upon stimulation.

The functional connectivity of CLIP-170 phosphorylation in MTOC repositioning was assessed. Fluorescence imaging of MTOCs and microtubules showed that an AMPK inhibitor, compound C, also disturbed microtubule frameworks and decentred the MTOC position. The relative expression of IL-2 in the presence of compound C decreased significantly compared to that in the absence of this compound, indicating that phosphorylation of CLIP-170 mediates both MTOC repositioning and full

activation of T cells upon stimulation.

2. CLIP-170 S312 phosphorylation is responsible for MTOC repositioning and T cell activation

To clarify the roles of CLIP-170 phosphorylation in MTOC repositioning, two human CLIP-170 mutants, a phosphomimetic S312D mutant and a phosphodeficient S312A mutant were generated. S312D mutation rescued the impaired MTOC centreing caused by AMPK inhibition. Whereas, the phosphodeficient S312A mutant showed disturbed microtubule frameworks and decentred the MTOC position. The relative IL-2 expression in cells with the S312A mutant decreased significantly compared to the wild-type cells. Thus, the effect of CLIP-170 Ser-312 phosphorylation was clearly shown.

To check the difference between the roles of CLIP-170 phosphorylation and T cell stimulation, MTOC distance and centreing fraction were quantitated. The MTOC distance from the contact surface of S312A mutant in stimulated cells was not significantly different from that of wild-type CLIP-170 in stimulated cells. In contrast, those of wild-type CLIP-170 in stimulated cells and in unstimulated cells showed a significant difference. The MTOC centreing fraction of S312A mutant in stimulated cells largely decreased compared with that of the wild-type in stimulated cells. These findings indicate that MTOC repositioning and full activation of T cells require both CLIP-170 phosphorylation and T cell stimulation.

3. Functional changes in dynamics and localisation of CLIP-170 and dynein

Finally, the functional relation between CLIP-170 phosphorylation and T cell stimulation was investigated using dual-colour TIRF live-cell imaging to visualise colocalisations and movement of CLIP-170 and dynein light chain (DLC). When the distribution of dynein was compared, stimulated wild-type CLIP-170 cells show dynein clusters accumulated in the inner area. CLIP-170 also localised in the same inner area, suggesting broad colocalisation. In case of stimulated S312A mutant, dynein is not accumulated in the inner area. These observations suggest that dynein molecules are anchored at the cell surface of the central region after T cell stimulation. Further quantification of the fractions of colocalised and non-colocalised clusters at the centre or periphery region revealed that coexistence of plus-end- and minus-end-directed dynein at the centre requires both T cell stimulation and CLIP-170 phosphorylation, and increased dynein relocation to the centre also requires both the stimulation and CLIP-170 phosphorylation.

Discussion and Conclusion: This study reports the role of CLIP-170 in regulating dynein localization analysed by simultaneous dual-colour fluorescence live-cell microscopy. Phosphorylated CLIP-170 is essential for dynein recruitment to the plus-end tracking. T cell stimulation increases dynein minus-end-directed movement. Both plus-end-and minus-end-directed translocation of dynein are necessary for dynein relocation to the immunological synapse. Based on these results, a model of MTOC repositioning that support the dynein-driven pulling mechanism is proposed.