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学 位 論 文 の 要 旨

論文題目

A study on the dynamin-2-dependent regulatory mechanisms of microtubule dynamics

(ダイナミン-２依存的微小管動態制御機構の解明に関する研究)

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要旨

Microtubules are intrinsically polar filaments composed of heterodimers of alpha- and beta-tubulins. The heterodimer subunits assemble/disassemble at the plus-ends of the microtubules, resulting in fast growth/shrinkage of the filaments and achieve microtubule dynamics. Microtubules which undergo no subunit exchange are stabilized, and their stability are regulated by some microtubule-binding proteins, such as CLASPs and Tau. In neurons, individual microtubule has both dynamic and stabilized region and the balance of their fractions contribute to maintain proper morphology. Hyperdynamic microtubules have been observed in many neurodegenerative diseases, such as Alzheimer's disease and amyotrophic lateral sclerosis. In contrast, increased microtubule stability triggers the progression of neurodegeneration in hereditary spastic paraplegia. Thus, the dysregulation of microtubule stability contributes to the progression of various diseases. Absolutely, the regulation of microtubule dynamics and stability is important for a variety of cellular processes, but the details are not yet fully understood.

Here, we studied a large GTPase, dynamin, which was firstly purified from bovine brain and has shown microtubule-binding properties. Three dynamins have been identified in mammals, dynamin-1, dynamin-2 and dynamin-3. Dynamin mutants contribute to various human diseases. For instance, many DNM2 mutations have been identified from subtypes of Charcot–Marie–Tooth (CMT) neuropathy, CMT2M, CMTDIB, and type 1 of centronuclear myopathy (CNM). Overexpression of a CMT disease-related dynamin-2 mutant, 551Δ3, induced the accumulation of stabilized microtubules in COS-7 cells, which was assessed by

quantifying the level of acetylated tubulin, a marker of lone-live microtubules. Furthermore, knock down of dynamin-2 also exhibited similar results, revealing a critical role of dynamin-2 in regulating microtubule stability. However, the mechanisms are still unclear.
In this study, our purpose is to explore the mechanisms of dynamin-2-dependent microtubule regulation. For this purpose, we investigated the intrinsic functions of dynamin-2 that involved in regulating microtubules.

Classical dynamin consists of five domains, including GTPase, middle, pleckstrin homology (PH), GTPase effector (GE), and proline-rich (PR) domains. During endocytosis, dynamin can spontaneously self-assemble into rings or helices around the neck of the clathrin-coated pits, realized by the strong interaction between the middle and GE domains within and between dynamin dimers. Then, GTP-binding and hydrolysis are required to drive a conformational change in dynamin, resulting in vesicle detachment from the plasma membrane. Therefore, GTPase activity, membrane-binding and self-assembly abilities are the intrinsic properties of dynamin. To examine the necessity of dynamin-2 domains and the related properties, we generated a heterozygous *DNM2* mutant cell line with depleted endogenous dynamin-2 protein and increased stabilized microtubules. We performed rescue experiments by expressing function-defective mutants of dynamin-2 in these heterozygous mutant HeLa cells and quantified the level of stabilized microtubules. First, we examined domain-deletion mutants, dynamin-2-ΔGTPase, -ΔPH, -ΔMiddle, -ΔGE and -ΔPR. The heterozygous mutant HeLa cells expressing dynamin-2-ΔGTPase, -ΔMiddle, -ΔPH, or -ΔGE remained at a high level of stabilized microtubules, while dynamin-2-ΔPR reduced the level of stabilized microtubules similar to dynamin-2-WT. This result indicated that the GTPase, middle, PH, and GE domains of dynamin-2 play vital roles in regulating microtubule stability. Previous study has shown that the PR domain at the C-terminus of dynamin-1 binds to microtubules *in vitro.* We observed the colocalization of GFP-dynamin-2-wild type (WT) /ΔPR and microtubules in HeLa cells. We found that wild type dynamin-2 formed filaments and almost colocalized with microtubules. In contrast, dynamin-2-ΔPR mutant showed a diffuse cytoplasmic pattern, indicating that PR domain contribute to microtubule-binding in cells and the microtubule regulation by dynamin-2 is indirect. Then, to examine the role of the GTPase activity of dynamin-2 on microtubule stability, we expressed a GTPase defective mutant, K44A, in heterozygous HeLa cells and assessed the level of stabilized microtubules. Stabilized microtubules in dynamin-2-K44A overexpressing heterozygous cells were reduced

to a normal level, suggesting that GTPase activity is not essential for microtubule regulation.

We also generated two unassembled mutants, dynamin-2-R361S and -I684K. Expression of these dynamin-2 mutants in heterozygous mutant cells failed to decrease the level of stabilized microtubules, indicating that self-assembling ability is required for microtubule regulation. In addition, expression of a membrane-binding defective mutant K535A reduced stabilized microtubules in heterozygous mutant cells. However, a CMT-related mutant K562E, which also shows impaired lipid affinity rescued abnormal stabilized microtubules in heterozygous mutant cells. Therefore, whether the membrane-binding ability is a crucial property for dynamin-2 on microtubule regulation is still a mystery and further studies are needed to clarify role of membrane-binding ability in microtubule regulation.

This study investigated that how dynamin-2 regulate microtubule stability, which is an unsolved question for more than a decade. We proved new evidence demonstrating that GTPase, middle, PH and GE domains are required for microtubule regulation, and the result of ΔPR mutant suggested an indirect regulatory function of dynamin-2 on microtubules. Additionally, we demonstrated that self-assembling ability, but not the GTPase activity of dynamin-2, plays a significant role in regulating microtubule stability. Therefore, we suggest that the regulatory mechanism of microtubule stability by dynamin-2 differs from that of endocytosis.