博士論文（要約）

Structural and Functional Analysis of Schizophrenia-related Microtubule-associated Proteins, KIF3 Complex and CRMP2

（統合失調症関連微小管結合蛋白質 KIF3 複合体及び CRMP2 の構造・機能解析）

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論文の内容の要旨

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Schizophrenia (SCZ) is a severe mental disorder with a ~1% prevalence worldwide. Microtubules (MTs), in support of MT-associated proteins (MAPs), provide essential scaffolds for neuronal structure and intracellular trafficking, abnormities of which are responsible for the neurodevelopmental deficits in SCZ. However, the lack of a clear elucidation of the casual effects regarding the molecular basis and mechanisms hinders our further understanding of the underlying pathogenic mechanism of SCZ as well as the development of fundamental effective drugs. Two MAPs, KIF3 and CRMP2, have been reported to be associated with the development of SCZ. The dysfunction of the KIF3-mediated transport of APC and CRMP2 was found to underlie the pathogenic pathway of SCZ, however, the biochemical and structural basis remains elusive. Enhanced carbonyl stress is a critical pathophysiology for SCZ, but little is known regarding the molecular pathogenesis. CRMP2 was identified as a major target of hyper-carbonyl modification in SCZ and the attenuation of carbonyl stress could compensate the local deficiency of CRMP2 resulting from the dysfunction of KIF3. These findings have underscored the critical role of CRMP2 in the development of SCZ, however, the relevant pathogenic pathway still remains unclear.

Here, in the first part of this thesis, I sought to explore the underlying molecular mechanism of SCZ-related KIF3-mediated cargo trafficking by determining the protein structures of KIF3 (KIF3A/B/KAP3) and KIF3-cargo complexes. The core regions of KIF3A and KIF3B necessary for forming the heterotrimeric KIF3 complex were identified by pull-down assays, and the KIF3 complex protein was successfully reconstituted. Further, the binding of APCARM with the reconstituted KIF3 complex was confirmed by pull-down and size exclusion chromatography (SEC) assays, and the stable KIF3-APCARM complex protein was obtained. The complex proteins were subsequently applied to crystallization trials and SEC-multi-angle light scattering, SEC-small angle X-ray scattering, cross-linking mass spectrometry and cryogenic electron microscopy assessments. Overall, these results uncovered the structure outlines, molecular assemblies and intermolecular interaction patterns of KIF3 and KIF3-APC

complexes, which provide crucial insights into our understanding of the working mechanism of the SCZ-related intracellular transport mediated by KIF3.

In the second part of this thesis, GLO1-deficient iPSCs were analyzed to uncover the missing link between the pathogenesis of SCZ and enhanced carbonyl stress. By immunoblotting and liquid chromatography-mass spectrometry (LC-MS) analysis, CRMP2 was identified to be the major protein target of hyper-carbonyl (AGE) modification in the patient-derived iPSCs with elevated carbonyl stress. Subsequently, AGE-modified sites of CRMP2 were detected using the LC-MS/MS technique using both iPSC-extracted and recombinant protein samples. Next, it was indicated by MT-bundling, MT-associating and MT-stabilizing assays that AGE modification can lead to the dysfunction of CRMP2. The further structural and biochemical analysis suggested that the dimeric and tetrameric interfaces of CRMP2, which are responsible for the reversibly transformative dynamic of CRMP2, may be disrupted by AGE modifications. More importantly, carbonylated CRMP2 (AGE-CRMP2) was observed to exhibit irreversible multimerization probably via crosslinked AGE modified residues, which led to the significant disruption of MT-associating activity of CRMP2. Collectively, these insights into the molecular mechanism of carbonyl stress-induced multimerization and dysfunction of CRMP2 explain the cellular developmental deficits of iPSCs with GLO1 deficiency and advance our understanding of the development of SCZ under elevated carbonyl stress, which could aid the development of new therapeutic strategies of SCZ.

In conclusion, the biochemical and structural findings validated the SCZ-related cargo binding of KIF3 in vitro and provided a possible structure model of the KIF3 tail-KAP3-APC complex. The results would advance our current understanding of the SCZ-related KIF3-mediated intracellular transport system with structural insights into the underlying molecular mechanism. Meanwhile, this study also provides the first direct evidence showing CRMP2 as a target of enhanced carbonyl stress in an SCZ subset, with insights into the mechanism of functional consequences of AGE-modified CRMP2 at an early developmental stage. Further structural analyses in solution and in situ, and physiological analyses in neurons and in vivo are expected. Collectively, these findings would advance our current understanding of the SCZ-related cytoskeletal dysfunction with molecular insights into the underlying mechanisms.