Structural Insights into a Novel Pathogenic Pathway of Schizophrenia under Carbonyl Stress

Enhanced carbonyl stress is a critical pathophysiology for multiple diseases, including schizophrenia, but little is known about the molecular pathogenesis. CRMP2, a crucial multifunctional regulatory protein that is highly expressed in human brain, was identified as a major target of hyper-carbonyl modification (AGE modification) in schizophrenia patient-derived iPS cells. Using a multimodal combination of iPS technology, cell biology, biochemistry, mass spectrometry and structural biology, we found that carbonylated CRMP2 is stacked in irreversible cross-linked multimer states via AGE modification and thereby results in neurodevelopmental deficits, which underlies the major pathogenic pathway of schizophrenia under carbonyl stress.

Neurodevelopmental impairments are considered to play crucial roles in the pathogenesis of schizophrenia, one of the most prevalent and serious psychiatric disorders. Oxidative stress is a significant risk factor for schizophrenia pathogenesis that leverages the generation of advanced glycation end products (AGEs) and is also called “carbonyl stress.” Enhanced carbonyl stress and deficiency of a central AGEs-scavenger enzyme (GLO1) reportedly underlie a subtype of schizophrenia, however, the causal effects and molecular pathogenesis remain elusive. CRMP2 is a microtubule (MT)-associated protein with various functions and reportedly plays a potential role in the etiopathogenesis of schizophrenia. Moreover, CRMP2 has been revealed to exhibit dynamic conformational transition among multimer, tetramer and monomer states for its specific function and regulation [1].

To unveil the missing link between development of schizophrenia and elevated carbonyl stress, patient-derived (with *GLO1* disruption) and *GLO1*-knockout iPS cells were constructed as cellular models, both of which exhibit enhanced carbonyl stress and significant neurodevelopmental deficits. Subsequently, CRMP2 was identified to be a major protein target of AGE modification by immunoblotting and LC-MS/MS analyses and about 60 AGE-modified sites were found to be widely distributed in the functional domains and two self-assembly interfaces (D-hook for dimerization and T-site for tetramerization) of CRMP2 [**Fig. 1(A)**]. Further MT-bundling, MT-binding, MT-turbidity and MT-stability assays indicated that AGE modification induced a significant loss of activity of CRMP2 [1].

Next, to investigate the structural mechanism for explaining how AGE modification impairs CRMP2 activity, unmodified and AGE-modified CRMP2 proteins were crystallized and the diffraction data were collected in the Photon Factory of KEK for structure determination. The final structures were determined at resolutions of 2.26 and 2.00 Å with R_{work}/R_{free} values of 17.1%/19.3% and 18.3%/20.4%, respectively (PDB codes: 6JV9 and 6JVB). In light of the structures, some significant AGE-induced structural changes were observed. First, an obvious discrepancy was found in the N-terminal area that comprises an AGE-targeted lysine in the T-site. Two β sheets (β2-β3) underwent sharp structural deflections via AGE modification, which probably results from AGE-induced changes of tetrameric packing [1]. Most importantly, in the AGE-CRMP2 structure, extra extended electron densities were observed for AGE-lysine pairs within the T-site as well as AGE-lysine residues on the outer surface of tetrameric CRMP2, indicating the AGE-induced formation of intermolecular cross-linking bridges which may impair the dynamic transformative conformation of CRMP2 and induce aberrant multimerization [**Fig. 1(B)**]. Next, high-resolution size exclusion chromatography [2, 3] and differential scanning calorimetry assays were performed to evaluate the dynamic size distribution and protein flexibility of AGE-CRMP2 in solution. The results showed that AGE-CRMP2 exhibited lower flexibility and a substantial increase of multimeric and aggregated forms, indicating that the dynamic equilibrium in reversibly formed CRMP2 complexes was disrupted by AGE modification [1]. Collectively, these structural and biochemical analyses revealed that AGE-CRMP2 is stacked in irreversible multimer states via AGE modification at D-hook, T-site and outer surface of tetrameric CRMP2, leading to the loss of its unique functions [**Fig. 1(C)**].

Taken together, this work provides direct evidence of the dysfunction of CRMP2 under enhanced carbonyl stress, explains the cellular developmental deficits of patients’ iPS cells, and provides the novel conclusion that AGE-CRMP2 multimerization is a major pathogenic pathway of schizophrenia under carbonyl stress [**Fig. 1(C)**], which could aid the development of new therapeutic strategies.

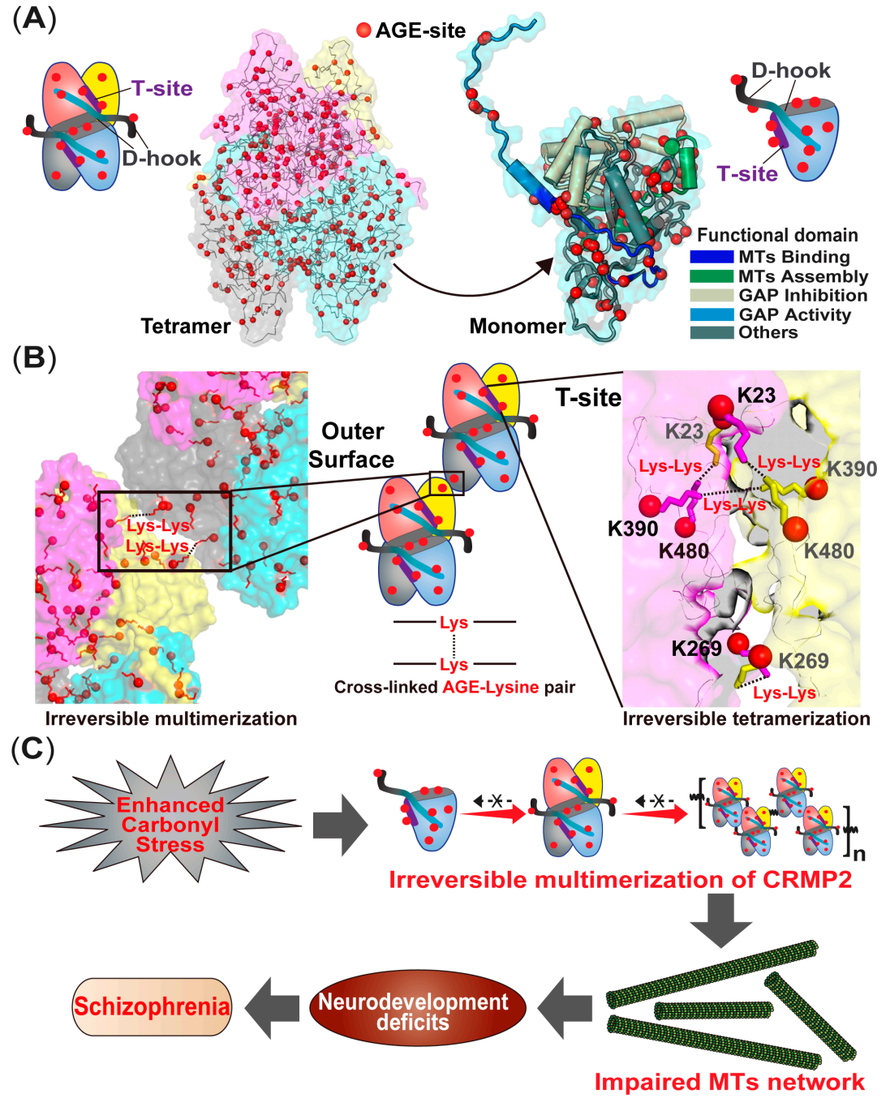

Figure 1: (A) The distribution of AGE-modification sites on functional domains and two self-assembly interfaces (D-hook and T-site) in the CRMP2 structure. **(B)** AGE modification results in the formation of cross-linked AGE-lysine bridges within a CRMP2 tetramer or among CRMP2 tetramers. **(C)** Model of the major pathogenic pathway of schizophrenia under carbonyl stress via CRMP2 multimerization.

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