RESEARCH HIGHLIGHT

SUMOylation of Fragile X Mental Retardation Protein: A Critical Mechanism of FMRP-Mediated Neuronal Function

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Recently, Khayachi *et al.* [1] showed that fragile X mental retardation protein (FMRP) is an active substrate of the small ubiquitin-like modifier (SUMO) pathway in neurons. FMRP SUMOylation is induced by the activation of metabotropic glutamate receptors (mGlu5Rs). FMRP SUMOylation is required for dissociating FMRP from dendritic RNA granules and controlling spine density and proper maturation. Mechanically, the SUMOylation process is triggered by the activation of mGlu5Rs, thereby contributing to maintaining FMRP-mediated neuronal function. In fact, some proteins that mediate synaptic plasticity, neurotransmitter release, and neuronal network formation [2–4], are mostly regulated by SUMOylation. Therefore, SUMOylation has emerged as an essential posttranslational modification in the nervous system. This novel discovery first provides evidence to support the idea that FMRP is a novel substrate of SUMOylation and acts as an essential regulator in the developing brain. Clearly, it also expands the biological ramifications of FMRP modification, since FMRP was previously supposed to be modulated by phosphorylation and dephosphorylation [2]. Together, their research reveals that the rapid SUMOylation of FMRP results from the activation of mGlu5Rs and

activity-dependent FMRP SUMOylation is a novel target for affecting neuronal functions.

SUMOylation, an enzymatic and dynamic posttranslational modification, is a process involving a member of the ubiquitin family. SUMOylation occurs when the SUMO protein is covalently attached to a lysine residue of a substrate protein. The process is mediated *via* a dedicated enzymatic cascade including SUMO E1 (the heterodimer SAE1 and SAE2), SUMO E2 conjugating enzyme (UBC9), and several E3 ligating enzymes [3]. Like most posttranslational modifications, SUMOylation is a reversible process; it is readily reversed by several SUMO-specific proteases in mammals. DeSUMOylation mainly removes SUMO protein from the substrates. SUMOylation and deSUMOylation are responsible for several important cellular functions, such as altering protein stability and regulating protein-protein interactions. Recent studies have shown that SUMO-modified proteins are present in the nucleus and SUMOylation plays a crucial role in regulating synaptic communication and brain development [4, 5]. Consequently, perturbations in neuronal SUMOylation are implicated in some pathological conditions.

A series of studies suggest that the SUMOylation pathway plays an important role in maintaining neuronal function *via* related signaling pathways (Fig. 1). PIASx is a SUMO E3 ligase that significantly inhibits MEF2 (myocyte enhancer factor 2)-dependent transcriptional activity in neurons. PIASx-MEF2 SUMOylation signaling is considered to be a key mechanism to promote dendritic claw differentiation and morphogenesis in the cerebellar cortex [6]. Dendritic claws are required for the formation of neural connectivity in brain development. In addition, the SUMOylation of MeCP2 (methyl CpG binding protein 2) by the E3 ligase PIAS1 is necessary for repressing the transcriptional function of MeCP2 and regulating synaptic

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Fig. 1 Involvement of the SUMOylation pathway in maintaining neuronal function.

density in the central nervous system [7]. Forkhead box P2 (FOXP2), an essential factor for language and speech development, has been identified as a SUMO target protein *via* another E3 ligase, PIAS3. FOXP2 SUMOylation plays a fundamental role in regulating the transcriptional activity of FOXP2 and controlling cerebellar motor function and vocal communication [8]. Consistently, FOXP1 SUMOylation at lysine 670 is required for proper dendritic morphogenesis and neuronal network formation in the brain *via* recruiting the co-repressor CtBP1 (C-terminal binding protein 1) [4]. Besides, the SUMOylation of Rab3-interactive molecule-1α (RIM1α), a major active zone protein for regulating neural plasticity, has a central impact on fast synaptic vesicle exocytosis and synaptic plasticity [5]. Syntaxin1A, one of SNARE proteins, is important to maintaining the balance of synaptic vesicle endo/exocytosis and driving neurotransmitter release through its SUMOylation [9]. All these results reveal a critical involvement of SUMOylation in maintaining neurodevelopmental processes ranging from synaptic plasticity and neurotransmitter release to neuronal network formation.

It has also been demonstrated that dysfunctions in neuronal SUMOylation play a potential role in contributing to some neurological disorders. Rett syndrome (RTT) is a severe neural developmental disorder caused by MeCP2 dysfunction [10]. Interestingly, some social behavioral deficits and synaptic plasticity abnormalities caused by RTT are rescued by MeCP2 SUMOylation, revealing a causative role of abnormal MeCP2 SUMOylation in RTT [11]. Alzheimer's disease (AD) is a well-known neurodegenerative disease, pathologically characterized by the deposition of amyloid precursor protein (APP). APP aggregation has been identified as a SUMOylated substrate

[12]. Studies have demonstrated impaired APP SUMOylation both in human AD brain tissue and the transgenic AD mouse model, while supplementing SUMOylation by transduction of the conjugating enzyme-Ubc9, can significantly rescue AD-induced cognitive deficits [13]. Together, these findings point out the contributions of SUMOylation impairments in neurological pathogenesis, such as RTT and AD.

To date, even though many modifications of FMRP have been reported to regulate neuronal function, a study of FMRP-targeted SUMOylation had never been published before. In detail, the finding that the SUMOylation occurs precisely at the N-terminal K88 and K130 and C-terminal K614 residues of FMRP is particularly important to deepen the understanding of FMRP-mediated neuronal function. Of note, only K88 and K130 in N-terminal lysines, but not C-terminal K614, are available for regulating dendritic spine density and maturation. The interesting data support the hypothesis that the N-terminal SUMOylation of FMRP is specifically critical for FMRP-mediated neuronal events. Recently, missense point mutations of FMR1 gene F126S and R138Q are regarded as a cause of fragile X syndrome (FXS) [14]. Of note, the point mutation R138Q is close to the active SUMO site K130 in FMRP. Thus, it is possible that mutations of FMRP near its SUMOylation site affect FMRP SUMOylation and so contribute to FXS etiology; this awaits testing in future studies. Therefore, further research work is needed to understand the association between known FMRP point mutations and its SUMOylation sites, as well as the effects of FMRP SUMOylation site mutation on FXS.

More specifically, the mGlu5R serves to drive FMRP SUMOylation. In fact, mGlu5Rs have been previously

reported to induce FMRP dephosphorylation and ubiquitination. Upon stimulation with an mGlu5R agonist, FMRP is rapidly dephosphorylated at a serine residue in its C-terminal; this is mediated by protein phosphatase 2A [2]. Meanwhile, recent evidence suggests that FMRP is also phosphorylated by casein kinase II at serine residue 499 (S499), even permitting secondary phosphorylation of FMRP [15]. Accordingly, there is a crosstalk between such protein modifications of FMRP, and this discovery adds further complexity to FMRP posttranslational modifications in neurons. Thus, future studies to assess the specific interactions between these modifications of FMRP and to shed further light on the activity-dependent regulation of FMRP by mGlu5Rs are urgently needed.

Additional explorations are needed to precisely clarify whether other FMRP modifications have an impact in FXS pathology. And future forward steps are needed to confirm the participation of FMRP SUMOylation defects in FXS patients as well as to identify the upstream and downstream signals to develop potential therapeutic approaches. Since FMRP loss could result in excessive activation of mGluR5s and further the FXS phenotype, the mGluR5 antagonist Fenobam has promise as a therapeutic strategy to cure FXS [16]. Thus, it is postulated that the combined pharmacotherapies of simultaneously targeting mGluR5s and FMRP-SUMOylation might provide a novel treatment option for FXS, but this needs further exploration.

Collectively, FMRP-SUMOylation is a critical mechanism of FMRP-mediated neuronal function such as synaptic plasticity, neurotransmitter release, and neuronal network formation. More noteworthy, SUMOylation abnormality in neurons plays a vital role in some neurodegenerative disorders, such as RTT and AD. Therefore, drugs targeted at abolishing abnormal SUMOylation may help to maintain normal neuronal functions and prevent the development of some neurodegenerative diseases.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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