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SUMO1 modification of 0N4R-tau is regulated by PIASx, SENP1, SENP2, and TRIM11

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ABSTRACT

Tau is a microtubule-associated protein that contributes to cytoskeletal stabilization. Aggregation of tau proteins is associated with neurodegenerative disorders such as Alzheimer's disease. Several types of posttranslational modifications that alter the physical properties of tau proteins have been identified. SUMOylation is a reversible modification of lysine residues by a small ubiquitin-like modifier (SUMO). In this study, we examined the enzymes that regulate the SUMOylation and deSUMOylation of tau in an alternatively spliced form, 0N4R-tau. Among SUMO E3 ligases, we found protein inhibitor of activated STAT (PIAS)xα and PIASxβ increase the levels of SUMOylated tau. The deSUMOylation enzymes sentrin-specific protease (SENP)1 and SENP2 reduced the levels of SUMO-conjugated tau. SUMO1 modification increased the level of phosphorylated tau, which was suppressed in the presence of SENP1. Furthermore, we examined the effect of tripartite motif (TRIM)11, which was recently identified as an E3 ligase for SUMO2 modification of tau. We found that TRIM11 increased the modification of both 2N4R- and 0N4R-tau by SUMO1, which was attenuated by mutation of the target lysine residue to arginine. These findings suggest that the expression and activity of SUMOylation regulatory proteins modulate the physical properties of tau proteins and may contribute to the onset and/or progression of tau-associated neurodegenerative disorders.

1. Introduction

SUMOylation is a posttranslational modification in which a small ubiquitin-like modifier (SUMO) is conjugated to lysine residues of target proteins. Such modifications lead to various changes in the physical properties of the target proteins that modulate their functions. For instance, modification changes the subcellular localization of target proteins, sensitivity to other modifications such as ubiquitination, and interaction with other proteins. Three major SUMO isoforms, SUMO1, SUMO2, and SUMO3, are known. SUMO2 and SUMO3 are 97 % identical and often referred to as SUMO2/3, while the identity between SUMO1 and SUMO2/3 is only 47 %. SUMO1 is usually conjugated to the target as a monomer, while SUMO2/3 can build poly-SUMO chain. SUMOylation is mediated by E1, E2, and E3 enzymes. E1, a heterodimer of SAE1 and SAE2, activates SUMO. Ubc9 is a SUMO-specific E2 enzyme that conjugates SUMO to target proteins. Although Ubc9 is sufficient for conjugation and substrate recognition, SUMO conjugation is mediated by various E3 ligases [1]. PIAS (protein inhibitor of activated STAT) family proteins function as SUMO E3 ligases. Four mammalian PIAS genes, *PIAS1*, *PIAS2* (*PIASx*), *PIAS3*, and *PIAS4* (*PIASy*), are ubiquitously expressed [2]. SUMOylation is also regulated by deSUMOylation enzymes that remove SUMOs from target proteins. Six SENP (sentrin-specific protease) family proteins (SENP1-3 and SENP5-7) have been identified [3]. They are mainly localized in the nucleus, and SENP1 and SENP2 are also found in extranuclear compartments and in the cytoplasm [4]. The balance between SUMOylation and deSUMOylation enzymes influences the SUMOylation rate.

Tau is a microtubule-associated protein predominantly expressed in the axons of neurons. Tau contributes to cytoskeletal stabilization by binding to microtubules through its microtubule-binding domain, which contains three or four repeats of the tubulin-binding motif. Aggregation of tau proteins is associated with neurodegenerative disorders such as Alzheimer's disease (AD). In AD brains, abnormally hyperphosphorylated tau forms intraneuronal neurofibrillary tangles (NFT), a hallmark of AD pathology. The human tau gene *MAPT* comprises 16 exons. Two N-terminal insertions encoded by exons 2 and 3 are

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associated with the subcellular localization of tau in neurons. Exon 10 encodes one of the microtubule-biding repeat domains [5]. In the adult human brain, six isoforms are generated by the alternative splicing of exons 2, 3, and 10. Among them, isoforms with both exons 2 and 3 (2N-tau) comprise only ~9 % of total tau, whereas isoforms without exon 3 (1N-tau) or both exons 2 and 3 (0N-tau) comprise ~54 % and 37 %, respectively [6]. Tau has 85 sites (45 serines, 35 threonines, and 5 tyrosines) that can be phosphorylated physiologically and/or pathologically in its longest splicing isoform (2N4R-tau) [7]. In addition to phosphorylation, other posttranslational modifications have been observed in tau, including ubiquitination, acetylation, methylation, and glycosylation [8].

Dorval and Fraser [9] showed that tau is modified by SUMO1 and, to a much lesser extent, by SUMO2 and 3. The SUMO1 modification site is lysine (K) 340 in 2N4R-tau, located in the SUMOylation consensus sequence **ψ**KX(E/D). SUMOylation of tau was increased in the presence of a phosphatase inhibitor, indicating that tau phosphorylation upregulates its SUMOylation. Luo et al. [10] further demonstrated that SUMOylation of tau promotes its hyperphosphorylation at AD-associated sites and reduces its solubility. They also showed that SUMO1 co-localizes with phosphorylated tau in the cortex and hippocampus of AD brains, indicating a positive association between tau phosphorylation and SUMOylation under pathological conditions. However, the regulation of this linkage remains unclear. Recently, Zhang et al. [11] reported that modification of tau with SUMO2 is directly mediated and promoted by TRIM11, a tripartite motif (TRIM) protein. They demonstrated that TRIM11 promoted the degradation and disaggregation of tau fibrils. These findings imply that SUMOylation plays diverse roles in controlling the physical properties of tau protein. To further understand the regulation of tau SUMOylation, in the present study, we identified enzymes that regulate SUMOylation and deSUMOylation of tau.

2. Materials and methods

2.1. Plasmids

pRK5-EGFP-Tau (0N4R) was a gift from Karen Ashe (Addgene plasmid # 46904) [12]. To construct pcDNA-FLAG-tau, tau cDNA was amplified by PCR with primers (5′-ACGATGACAAGCTTGGCATGGACG AGCTGTACAAG-3′ and 5′- GATATCTGCAGAATTCTCGACTCACAA ACCCTGCTT-3′) and inserted between the *Kpn*I and *Eco*RI sites of pcDNA3.1(+) with FLAG sequence (ACCATGGATTACAA GGACGACGATGAC between *Nhe*I and *Hin*dIII sites) by In-Fusion Cloning (Takara Bio, Shiga, Japan). K282R mutation was introduced in tau by site-directed mutagenesis using the primers (5′-GGTGGCC AGGTGGAAGTAAGATCTGAGAAGCTTGAC-3′ and 5′- GTCEAGCTT CTCAGATCTTACTECCACCTCGCCACC-3′). K44R–0N4R-tau and K44 R/K282R–0N4R-tau were generated by introducing K44R mutation using primers (5′-CGGACGCTGGCCTGAGAGCTGAAGAAGCAGG -3′ and 5′-CCTGCTTCTTCAGCTCTCAGGCCAGCGTCCG -3′). 2N4R-tau was generated by replacing the 2N-containing region with the corresponding region in 0N4R-tau. 2N region was obtained by RT-PCR with total RNA isolated from SH-SY5Y cells using primers (5′- AAGGATCCATGGCT-GAGCCCCGCCAGGA -3′ and 5′- GCTGTGGTTCCTTCTGGGATC -3′). *Nru*I site was introduced in K44R–0N4R-tau using primers (5′- TCGCGAA-GAAGCAGGCATTGGAGAC -3′ and 5′- CTCAGGCCAGCGTCCGTGTCACC -3′) and N-terminal region was replaced with 2N region between *Bam*HI and *Nru*I sites. K340R mutation was introduced in 2N4R-tau by site-directed mutagenesis using the primers for K282R mutation. K44R–2N4R-tau and K44R/K282R–2N4R-tau were generated by introducing K44R mutation using primers (5′- 5′- CGGACGCTGGCCTGAGA-GAATCTCCCCTGC -3′ and 5′- GCAGGGGAGATTCTCTCAGGCCA GCGTCCG -3′). The plasmid expressing HA-tagged SUMO1 was described in Niikura et al. [13]. FLAG-mPIAS1, FLAG-mPIAS3, FLAG-hPIASy, FLAG-hPIASxα, and FLAG-hPIASxβ were gifts from Ke Shuai (Addgene plasmids # 15206, 15207, 15208, 15209, 15210, respectively) [14–17]. FLAG-SENP1, FLAG-SENP2, RGS-SENP3, and FLAG-SENP6 were gifts from Edward Yeh (Addgene plasmids # 17357, 18047, 18048, 18065, respectively) [17–20]. The His-tagged SENP plasmids, SENP1 and SENP2, were described in Maruyama et al. [21]. pEGFP-TRIM11 was generated by inserting the mouse TRIM11 fragment [22] into the pEGFP-C3 vector (Clontech) between the *Sma*I and *Xho*I sites.

2.2. Cell culture, transfection, and immunoprecipitation

HEK293 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % fetal bovine serum (FBS), 50 units/mL of penicillin, and 50 μg/mL of streptomycin (Thermo Fisher Scientific, Waltham, MA, USA). Cells (7×10^5 cells/dish) were seeded into 60-mm dishes and transfected with plasmids expressing FLAG-tagged or enhanced green fluorescent protein (EGFP)-fused tau (wild-type [wt] or mutant), HA-tagged SUMO1, or His-tagged or FLAG-tagged SENP/PIAS by lipofection (Lipofectamine 3000, Thermo Fisher Scientific). The plasmid DNAs were mixed at a ratio of 3:1:1 for tau: SUMO: SENP/PIAS. Immunoprecipitation with anti-FLAG antibody was performed as described in Niikura et al. [13]. For immunoprecipitation of EGFP-fused tau proteins, anti-GFP antibody (012–20461, Wako Pure Chemical Industries, Osaka, Japan) and protein G-Sepharose (GE Healthcare, Chicago, IL, USA) were used. For immunoblot analysis of phosphorylated tau, transfected cells were lysed with lysis buffer (20 mM Tris-HCl [pH 7.4], 150 mM NaCl, 1 % Triton X-100, 0.1 % SDS, 1 % sodium deoxycholate, 1 mM EDTA, 1 mM N-ethylmaleimide [NEM]) containing phosphatase inhibitors (PhosSTOP; Roche Diagnostics, Indianapolis, IN, USA) and protease inhibitors (cOmplete; Roche Diagnostics, Indianapolis, IN, USA). Cell debris was removed by centrifugation, and the clarified cell lysates (15 mg of protein) were subjected to immunoblot analysis.

2.3. Immunoblot analysis

Protein samples were separated by SDS-PAGE and transferred to polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA). The membranes were blocked with 5 % skim milk in TBS-T (20 mM Tris-HCl [pH 7.6], 136 mM NaCl, and 0.1 % Tween 20) and incubated with horseradish peroxidase (HRP)-conjugated primary antibodies or non-conjugated primary antibodies and HRP-conjugated secondary (anti-mouse or anti-rabbit) antibodies (7076S, 7074S, Cell Signaling Technologies, x5000), followed by detection with an enhanced chemiluminescence reagent (Clarity Western ECL Substrate, Bio-Rad, Hercules, CA, USA). The primary antibodies used were HRP-conjugated anti-HA (12013819001, Roche, x2000), HRP-conjugated anti-FLAG (A8592, Sigma, St. Louis, MO, USA, x2000), HRP-conjugated anti-actin (A3854, Sigma, x20,000), anti-phosphorylated tau (S396) (ab109390, abcam, x5000), anti-GFP (012–20461: Wako, x2000, ab15272: Clontech, x5000), anti-His (D291: MBL, x5000, 010–23181: Wako, x2000), anti-GSK3β (9315, Cell Signaling, x1000), anti-pGSK3β (S9) (9323, Cell Signaling, x2000), and anti-acetylated lysine (ab80178, abcam, x1000). Signal intensity was measured using Gene Tools (Syngene, Cambridge, UK).

2.4. Statistical analysis

The data are expressed as the mean ± SD. Multiple-group comparisons were performed using the One-way ANOVA followed by Tukey's multiple comparisons test. Statistical analyses were performed using Prism9 (GraphPad, La Jolla, CA, USA).

3. Results

3.1. 0N4R-tau is modified by SUMO1

Previous reports have demonstrated the SUMOylation of 2N4R-tau at K340 [9,10]. In this study, we used an alternative splice variant, 0N4R-tau, because it is more abundant than the 2N4R variant in the adult CNS [23]. First, we tested whether SUMOylation occurred in this tau variant (Fig. S1). HEK293 cells were transfected with plasmids expressing FLAG-tagged tau and HA-tagged SUMO1, and FLAG-tau proteins were immunoprecipitated with an anti-FLAG antibody. In the precipitates, an anti-HA antibody detected a band at approximately 85 kDa, which was also detected by an anti-FLAG antibody in the input samples (Fig. S1 A, B). 0N4R-tau was detected at approximately 60 kDa and the calculated molecular weight of SUMO1 is 12 kDa. Thus, the 85 kDa SUMOylated tau may have been modified by SUMO1 at 2 sites or had other modifications, such as phosphorylation. We also tested a tau K282R mutant (tau-KR, tau with a substitution of lysine 282 with arginine), which corresponds to K340R in the 2N4R variant. The intensity of HA-positive bands was significantly reduced, but not completely diminished, in tau-KR (Fig. S1A). The immunoprecipitates were boiled before performing SDS-PAGE. As a previous report explained that samples should not be boiled to detect tau SUMOylation [10], we examined the difference in sample preparation. The immunoprecipitates were divided into two groups, and one group was boiled for 10 min. We did not detect a significant difference between boiled and non-boiled

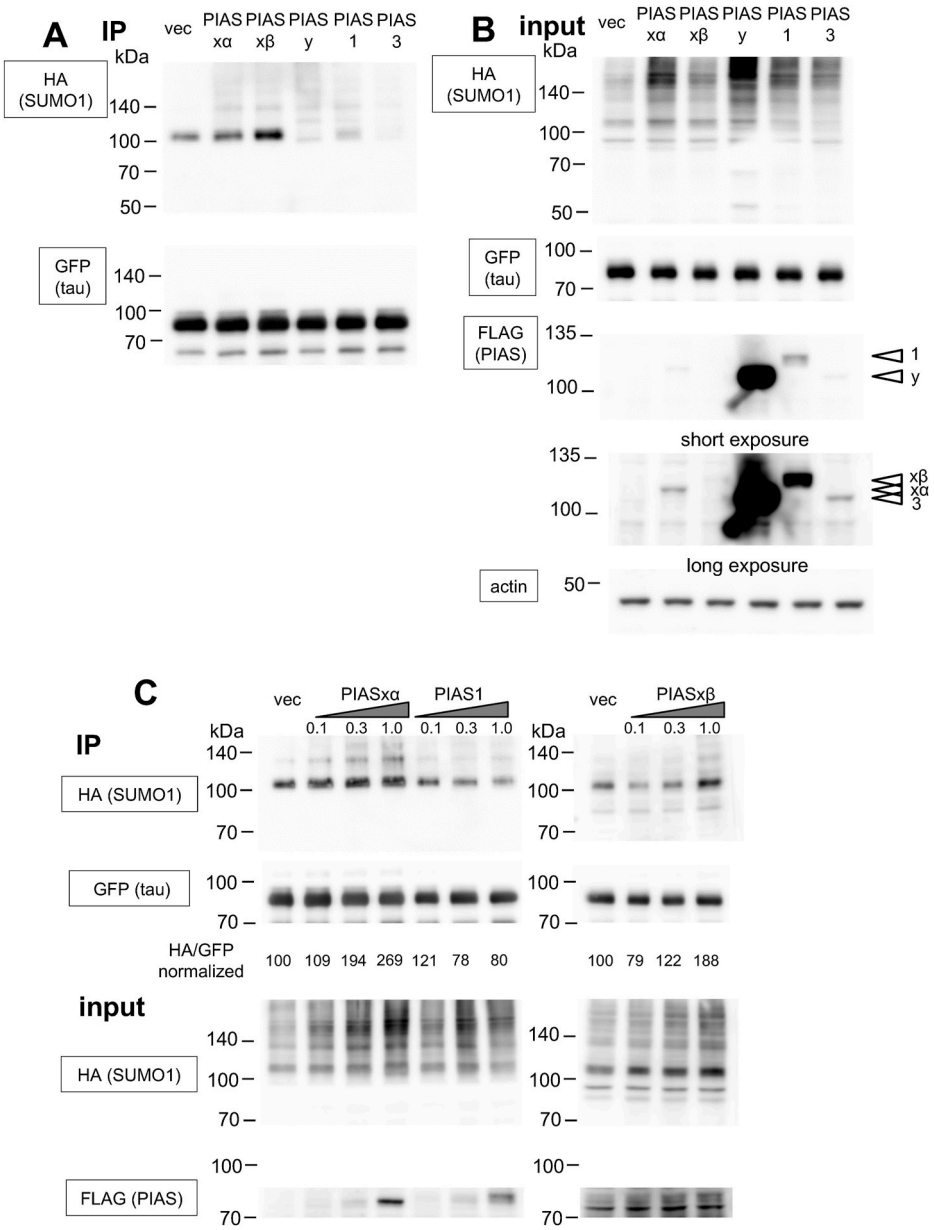

Fig. 1. Effect of PIAS proteins on 0N4R-tau SUMOylation

HEK293 cells were co-transfected with plasmids expressing EGFP-fused tau, HA-tagged SUMO1, and FLAG-tagged PIAS1, PIASxα, PIASxβ, PIAS3, or empty vector (vec). Cell lysates were immunoprecipitated with an anti-GFP antibody. Immunoprecipitate (IP) (**A**) and input lysate (input) (**B**) samples were subjected to immunoblotting with anti-HA, anti-FLAG, anti-GFP, and anti-actin antibodies. In B, arrowheads indicate the position of each PIAS protein band. (**C**) Different amounts (0.1, 0.3, 1.0 μg/sample) of FLAG-tagged PIAS plasmids were used. Immunoprecipitate (IP) (upper two panels) and input lysate (input) (lower two panels) samples were subjected to immunoblotting with anti-HA, anti-FLAG, and anti-GFP antibodies. The band intensity of the samples was measured, the value of HA was normalized to that of GFP, and then the values relative to the vector control (=100) were calculated and shown.

samples and observed both SUMOylated and total tau (Fig. S1C). These results indicate that 0N4R-tau can be SUMOylated at the same lysine residue as 2N4R-tau.

3.2. Effects of PIAS proteins on tau SUMOylation

SUMOylation E3 enzymes facilitate SUMO conjugation to target proteins and exhibit substrate selectivity. We examined which PIAS proteins and SUMO E3 ligases are involved in tau SUMOylation. We co-expressed FLAG-tagged PIAS proteins, EGFP-fused tau, and HA-tagged SUMO1, and immunoprecipitated tau with an anti-GFP antibody. We detected increased levels of SUMOylated tau in the presence of PIASxα and PIASxβ (Fig. 1A). On the other hand, global SUMOylation was increased in the presence of all types of PIAS proteins (Fig. 1B). PIAS proteins were detected with an anti-FLAG antibody; however, their expression levels varied. To further confirm the effect of PIASxα and PIASxβ, we performed a dose-response experiment (Fig. 1C). PIASxα and PIASxβ induced a dose-dependent increase in the levels of SUMOylated tau.

3.3. Effects of SENP proteins on tau SUMOylation

Next, we examined which SENP proteins function as deSUMOylation enzymes for tau. EGFP-fused tau was co-expressed with HA-tagged SUMO1 and FLAG-tagged SENP proteins and immunoprecipitated with an anti-GFP antibody. SENP1, 2, and 6 but not SENP3 reduced the levels of SUMO1-conjugated tau (Fig. 2A and B). We also performed a similar experiment using FLAG-tagged tau and His-tagged SENPs (Fig. S2) and found that the effect of SENP6 was insignificant. Since the effects of SENP1 and SENP2 were significant, we examined their dose-response effects using 6xHis-tagged SENPs (Fig. 2C). Both SENP1 and SENP2 reduced the levels of SUMO-conjugated tau in a dose-dependent manner (Fig. 2D).

Next, we examined the intracellular localization of tau and PIAS/SENP proteins. EGFP-fused tau was co-expressed with FLAG-tagged PIAS or SENP proteins in HEK293 cells, and the cells were stained with an

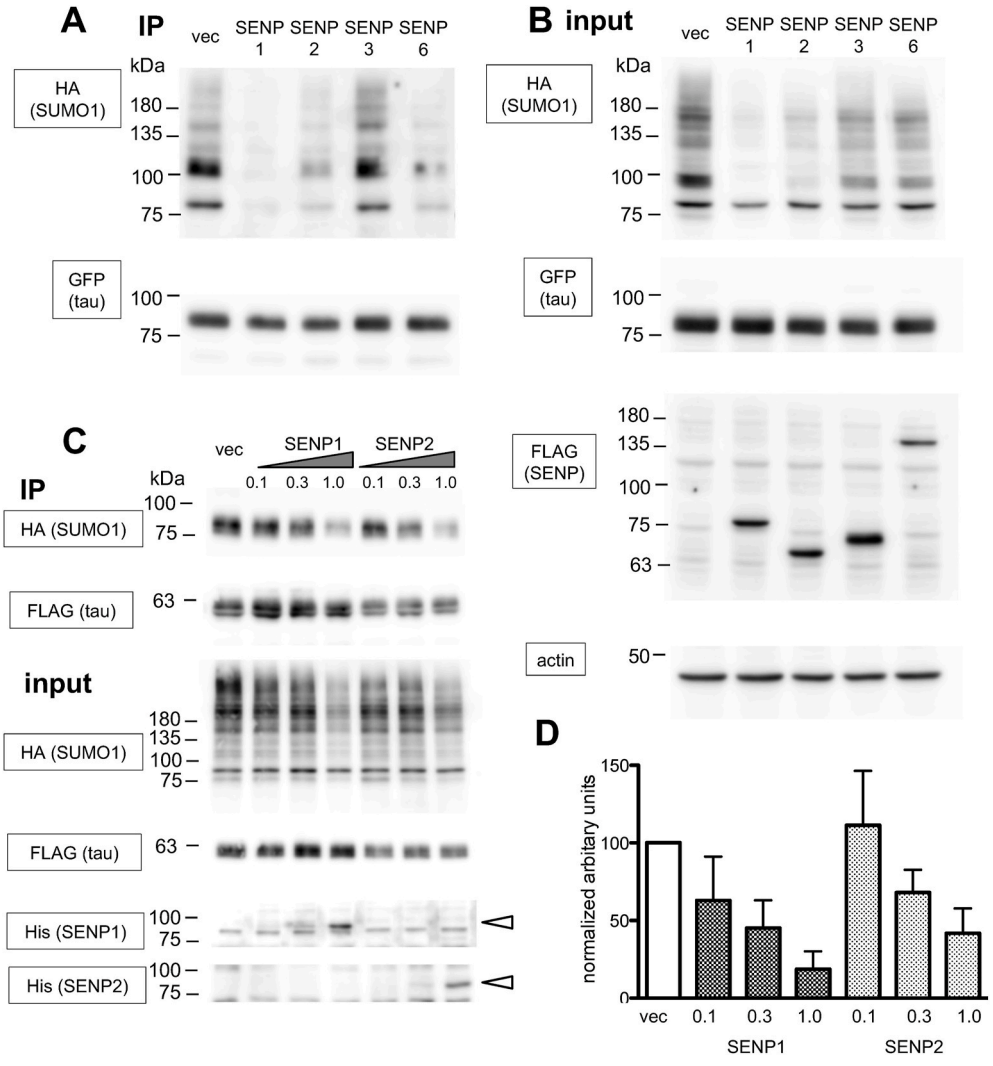

Fig. 2. Effect of SENP proteins on 0N4R-tau SUMOylation
(A) HEK293 cells were co-transfected with plasmids expressing EGFP-fused tau, HA-tagged SUMO1, and His-tagged SENP1, SENP2, SENP3, SENP6, or empty vector (vec). Cell lysates were immunoprecipitated using an anti-GFP antibody. **(B)** FLAG-tagged SENP proteins were used for the same experiment shown in A. Samples were subjected to immunoblotting with anti-HA, anti-FLAG, anti-GFP, and anti-actin antibodies. **(C)** HEK293 cells were co-transfected with plasmids expressing FLAG-tagged tau, HA-tagged SUMO1, and different amounts (0.1, 0.3, 1.0 μg/sample) of His-tagged SENP1 or SENP2. Immunoprecipitate (IP) (upper two panels) and input lysate (input) (lower four panels) samples were subjected to immunoblotting with anti-HA, anti-FLAG, and anti-His antibodies. Arrowheads indicate the position of each SENP protein band. **(D)** The band intensity of the samples was measured, the value of HA was normalized with that of FLAG, and then the values relative to vector control (=100) were calculated and shown. N = 4.

anti-FLAG-tagged primary antibody and Alexa Fluor568-conjugated secondary antibody (Fig. S3). Tau was detected in the cytoplasm. SENP1 was detected in the cytoplasm and overlapped with tau, whereas SENP2, SENP3, and SENP6 were found in the nucleus (Fig. S3A). All PIAS proteins, except PIASy, were observed in the nucleus (Fig. S3B). PIASy was detected in both the nucleus and cytoplasm and overlapped with tau distribution.

3.4. Effects of TRIM11 on tau SUMOylation

Recently, it was reported that TRIM11 directly promotes tau modification by SUMO2 [11]. Since tau is also modified by SUMO1, we examined whether TRIM11 affected SUMO1 modification of 0N4R-tau (Fig. 3). FLAG-tagged tau was co-expressed with HA-tagged SUMO1 and EGFP-fused TRIM11 proteins and immunoprecipitated with an anti-FLAG antibody. In the precipitates, the anti-HA antibody detected a band at approximately 85 kDa, which was also detected with an anti-FLAG antibody. The band was diminished in the presence of K282R with or without K44R mutations, confirming that SUMO1 was conjugated mainly to the lysine residue at position 282. The band intensity increased in the presence of TRIM11, indicating that TRIM11 enhanced the modification of 0N4R-tau by SUMO1. We also examined the effect of TRIM11 on the SUMOylation of 2N4R-tau (Fig. S4). Similar to 0N4R-tau, SUMOylation of 2N4R-tau was increased in the presence of TRIM11, which was largely attenuated by the K340R mutation. These results suggest that TRIM11 functions as an E3 ligase for SUMO1 modification of tau.

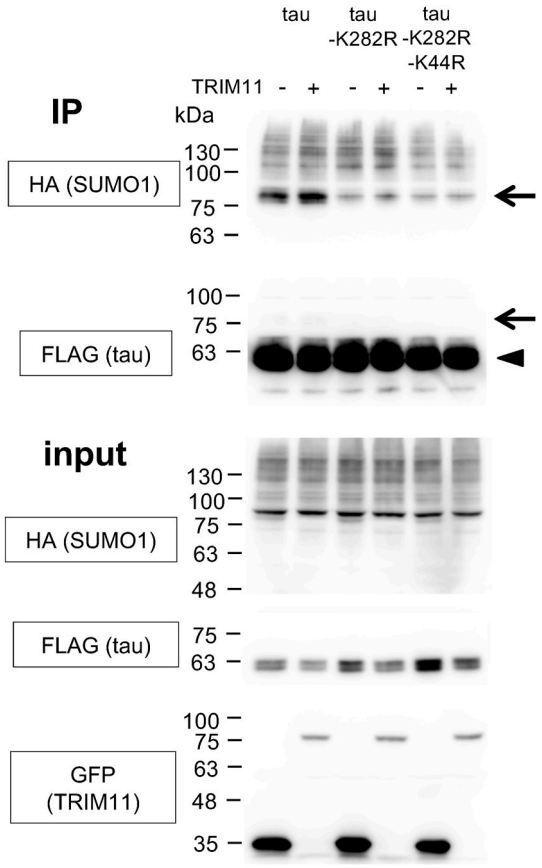

Fig. 3. Effect ofTRIM11 on 0N4R-tau SUMOylation
HEK293 cells were co-transfected with plasmids expressing FLAG-tagged tau, HA-tagged SUMO1, EGFP-fused TRIM11, or empty vector (vec). Cell lysates were immunoprecipitated with an anti-FLAG M2 antibody. Immunoprecipitate (IP) (upper two panels) and input lysate (input) (lower three panels) samples were subjected to immunoblotting with anti-HA, anti-FLAG, and anti-GFP antibodies. The arrows and arrowhead indicate the positions of SUMOylated and non-SUMOylated tau, respectively.

3.5. Effect of SUMOylation on tau phosphorylation and acetylation

We then investigated how tau SUMOylation affects other post-translational modifications. Luo et al. (2014) reported elevated phosphorylation levels in 2N4R-tau when SUMOylation was enhanced [10]. Thus, we examined the phosphorylation of 0N4R-tau at S338 (S396 in 2N4R-tau), a phosphorylation site associated with AD pathology and one of the targets of GSK3β phosphorylation of tau. In this experiment, we additionally expressed Ubc9, E2 enzyme, to enhance SUMOylation reaction. Increased band intensity of SUMOylated tau was detected using an anti-phosphorylated S396 antibody when SUMO1 and Ubc9 were co-expressed (Fig. 4A). In the presence of SENP1, the band intensity was attenuated. Quantitative analysis showed that the amount of phosphorylated tau was increased in correlation with SUMOylated tau protein levels (Fig. 4B). However, the expression and phosphorylation levels of GSK3β protein were not changed (Fig. 4B), suggesting that the overexpression of SUMO1 and Ubc9 did not affect GSK3β expression and activity.

In addition to phosphorylation, tau undergoes several modifications. Lysine residues are the targets of both acetylation and SUMOylation. Thus, we examined whether SUMOylation affected acetylation levels (Fig. S5). Anti-acetylated lysine antibody detected tau protein mainly in the non-SUMOylated form, suggesting that acetylation does not preferentially occur in the SUMOylated tau. The band intensity of the non-SUMOylated form did not change in the presence of SUMO1 or Ubc9. The band intensity was also unaffected in tau with the K282R mutation in the presence or absence of SUMO1 and Ubc9, suggesting that SUMOylation does not affect the overall acetylation status of tau.

4. Discussion

In this study, we showed that SUMO1 modification of 0N4R-tau was promoted by PIASx and decreased by SENP1 and SENP2 (Figs. 1 and 2) and that SUMOylated tau was phosphorylated at AD pathology-associated site (Fig. 4). This is consistent with previous findings using 2N4R-tau, which demonstrated that tau modification by SUMO1 increased the phosphorylation and reduced the solubility of tau [10] and that SUMO1 colocalized with phosphorylated tau aggregates [24]. We also showed that TRIM11 promotes SUMO1 modification of tau (Fig. 3). It has been reported that TRIM11 functions as an E3 ligase for tau modification by SUMO2 and promotes the solubilization and disaggregation of tau [11]. These findings suggest that modifications in SUMO1 and SUMO2 have opposite effects on tau by promoting or suppressing aggregation. Similar findings were reported for huntingtin, which is associated with Huntington's disease. Modification with SUMO1 and SUMO2 increases the solubility and aggregation of huntingtin, respectively [25,26]. Thus, it is assumed that the selection of SUMO type for modification determines tau aggregation. In the cortex of wild-type mice, global protein SUMOylation, both with SUMO1 and SUMO2/3, does not change from 2 to 6 months of age [21,27] but significantly decreases from 6 to 18 months of age [28]. Interestingly, global SUMOylation in synaptosomes increased with SUMO1 but decreased with SUMO2/3 up to 18 months of age [28]. These findings imply that the SUMOylation status may differ by cell type (neurons or glial cells) and/or intracellular location within neurons. Thus, it is speculated that the local regulation of SUMOylation in neurons may affect tau aggregation during aging.

We identified PIASx as an enhancer of tau SUMOylation (Fig. 1). Tau-SUMO1 fusion protein showed a propensity for tau aggregation [29]. PIASx (PIAS2) promotes SUMO1 modification of α-synuclein associated with Parkinson's disease, which results in aggregation of α-synuclein by blocking ubiquitination [30]. PIAS2 expression (mRNA and protein) is upregulated in the neurons of patients with Parkinson's disease

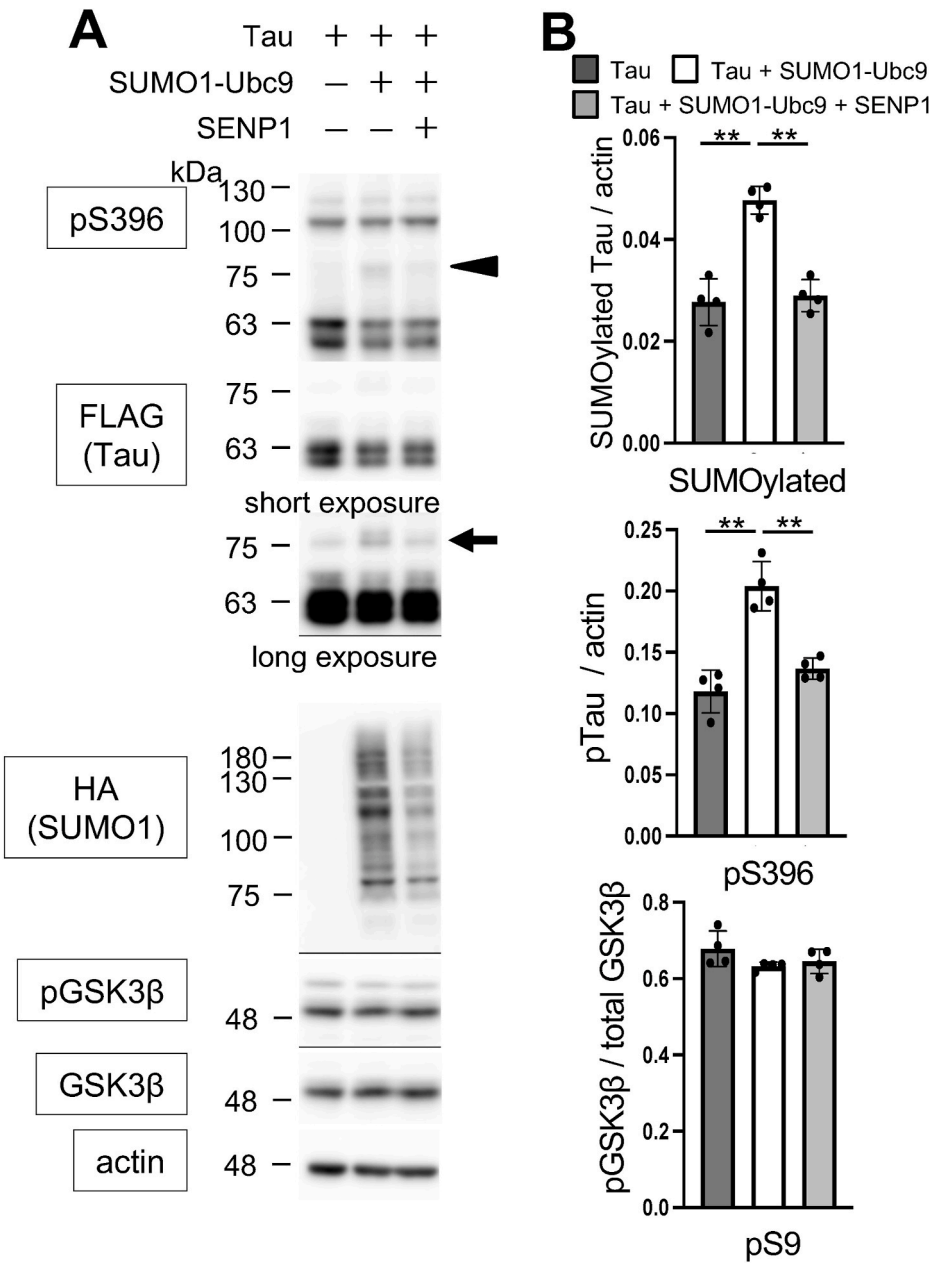

Fig. 4. Effect of SUMOylation on tau phosphorylation
HEK293 cells were co-transfected with plasmids expressing FLAG-tagged 0N4R-tau, HA-tagged SUMO1, myc-tagged Ubc9, His-tagged SENP1, or empty vector (vec). Cell lysates were subjected to immunoblotting with anti-phosphoS396-tau, anti-FLAG, anti-HA, anti-phosphoS9-GSK3β, anti-GSK-3β, and anti-actin antibodies. Representative blots are shown in **A**. Arrowhead and arrow indicate the 85 kDa (SUMOylated and phosphorylated tau) and 85 kDa (SUMOylated tau) bands, respectively. Quantitative results are shown in **B**. (n = 4). Band intensities of SUMOylated tau (SUMOylated) and phosphorylated tau (pS396) were normalized to that of actin. The band intensity of phosphorylated GSK3β (pS9) was normalized to that of total GSK3β.

compared to healthy controls [30,31], suggesting an association between PIASx and the progression of Parkinson's disease. Similarly, PIASy increases SUMOylation and enhances aggregation of superoxide dismutase 1 (SOD1) mutants associated with amyotrophic lateral sclerosis [32]. Therefore, increased PIASx levels may be a common cause of protein aggregation-associated neuronal diseases, including tau-linked disorders. SUMOylation and aggregation of SOD1 mutants are reduced by SENP1. In this study, we also found that SENP1 reduced SUMOylation of tau (Fig. 2). Oxidative stress increases SENP1 protein levels in primary cortical neurons [33]. Thus, it is assumed that aging and/or disease-associated oxidative stress may cause changes in the SUMOylation status of disease-linked proteins, leading to the progression of neurodegeneration. Notably, SENP family proteins function in both the deconjugation of SUMOs from target proteins and the maturation of SUMOs. A disturbance in the delicate balance of SENP expression may cause neurodegenerative disorders.

CRediT authorship contribution statement

Harmony Wada: Investigation, Formal analysis. **Takuma Maruyama:** Investigation. **Takako Niikura:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2024.101800.

References

[1] H.M. Chang, E.T.H. Yeh, Sumo: from bench to bedside, Physiol. Rev. 100 (2020) 1599–1619, https://doi.org/10.1152/physrev.00025.2019.
[2] M.M. Rytinki, S. Kaikkonen, P. Pehkonen, T. Jääskeläinen, J.J. Palvimo, PIAS proteins: pleiotropic interactors associated with SUMO, Cell. Mol. Life Sci. 66 (2009) 3029–3041, https://doi.org/10.1007/s00018-009-0061-z.
[3] R.T. Hay, SUMO-specific proteases: a twist in the tail, Trends Cell Biol. 17 (2007) 370–376, https://doi.org/10.1016/j.tcb.2007.08.002.
[4] J.M. Henley, T.J. Craig, K. a Wilkinson, Neuronal SUMOylation: mechanisms, physiology, and roles in neuronal dysfunction, Physiol. Rev. 94 (2014) 1249–1285, https://doi.org/10.1152/physrev.00008.2014.
[5] M. Goedert, M.G. Spillantini, Ordered assembly of tau protein and neurodegeneration, Adv. Exp. Med. Biol. 1184 (2019) 3–21, https://doi.org/10.1007/978-981-32-9358-8_1.
[6] Y. Wang, E. Mandelkow, Tau in physiology and pathology, Nat. Rev. Neurosci. 17 (2016) 5–21, https://doi.org/10.1038/nrn.2015.1.
[7] D.P. Hanger, B.H. Anderton, W. Noble, Tau phosphorylation: the therapeutic challenge for neurodegenerative disease, Trends Mol. Med. 15 (2009) 112–119, https://doi.org/10.1016/j.molmed.2009.01.003.
[8] L. Martin, X. Latypova, F. Terro, Post-translational modifications of tau protein: implications for Alzheimer's disease, Neurochem. Int. 58 (2011) 458–471, https://doi.org/10.1016/j.neuint.2010.12.023.
[9] V. Dorval, P.E. Fraser, Small ubiquitin-like modifier (SUMO) modification of natively unfolded proteins tau and α-synuclein, J. Biol. Chem. 281 (2006) 9919–9924, https://doi.org/10.1074/jbc.M510127200.
[10] H. Bin Luo, Y.Y. Xia, X.J. Shu, Z.C. Liu, Y. Feng, X.H. Liu, G. Yu, G. Yin, Y.S. Xiong, K. Zeng, J. Jiang, Y.K. Keqiang, X.C. Wang, J.Z. Wang, SUMOylation at K340 inhibits tau degradation through deregulating its phosphorylation and ubiquitination, Proc. Natl. Acad. Sci. U.S.A. 111 (2014) 16586–16591, https://doi.org/10.1073/pnas.1417548111.
[11] Z.Y. Zhang, D.S. Harischandra, R. Wang, S. Ghaisas, J.Y. Zhao, T.P. McMonagle, G. Zhu, K.D. Lacuarta, J. Song, J.Q. Trojanowski, H. Xu, V.M.Y. Lee, X. Yang, TRIM11 protects against tauopathies and is down-regulated in Alzheimer's disease, Science (80) (2023) 381, https://doi.org/10.1126/science.add6696.
[12] B.R. Hoover, M.N. Reed, J. Su, R.D. Penrod, L.A. Kotilinek, M.K. Grant, R. Pitstick, G.A. Carlson, L.M. Lanier, L.-L. Yuan, K.H. Ashe, D. Liao, Tau mislocalization to dendritic spines mediates synaptic dysfunction independently of neurodegeneration, Neuron 68 (2010) 1067–1081, https://doi.org/10.1016/j.neuron.2010.11.030.
[13] T. Niikura, Y. Kita, Y. Abe, SUMO3 modification accelerates the aggregation of ALS-linked SOD1 mutants, PLoS One 9 (2014) e101080, https://doi.org/10.1371/journal.pone.0101080.
[14] T. Arora, B. Liu, H. He, J. Kim, T.L. Murphy, K.M. Murphy, R.L. Modlin, K. Shuai, PIASx is a transcriptional Co-repressor of signal transducer and activator of transcription 4, J. Biol. Chem. 278 (2003) 21327–21330, https://doi.org/10.1074/jbc.C300119200.
[15] B. Liu, M. Gross, J. ten Hoeve, K. Shuai, A transcriptional corepressor of Stat1 with an essential LXXLL signature motif, Proc. Natl. Acad. Sci. USA 98 (2001) 3203–3207, https://doi.org/10.1073/pnas.051489598.
[16] C.D. Chung, J. Liao, B. Liu, X. Rao, P. Jay, P. Berta, K. Shuai, Specific inhibition of Stat3 signal transduction by PIAS3, Science 278 (1997) 1803–1805, https://doi.org/10.1126/science.278.5344.1803.
[17] B. Liu, J. Liao, X. Rao, S.A. Kushner, C.D. Chung, D.D. Chang, K. Shuai, Inhibition of Stat1-mediated gene activation by PIAS1, Proc. Natl. Acad. Sci. USA 95 (1998) 10626–10631, https://doi.org/10.1073/pnas.95.18.10626.
[18] H. Dou, C. Huang, M. Singh, P.B. Carpenter, E.T.H. Yeh, Regulation of DNA repair through deSUMOylation and SUMOylation of replication protein A complex, Mol. Cell. 39 (2010) 333–345, https://doi.org/10.1016/j.molcel.2010.07.021.
[19] L. Gong, E.T.H. Yeh, Characterization of a family of nucleolar SUMO-specific proteases with preference for SUMO-2 or SUMO-3, J. Biol. Chem. 281 (2006) 15869–15877, https://doi.org/10.1074/jbc.M511658200.
[20] X. Kang, Y. Qi, Y. Zuo, Q. Wang, Y. Zou, R.J. Schwartz, J. Cheng, E.T.H.H. Yeh, SUMO-specific protease 2 is essential for suppression of polycomb group protein-mediated gene silencing during embryonic development, Mol. Cell. 38 (2010) 191–201, https://doi.org/10.1016/j.molcel.2010.03.005.
[21] T. Maruyama, Y. Abe, T. Niikura, SENP1 and SENP2 regulate SUMOylation of amyloid precursor protein, Heliyon 4 (2018) e00601, https://doi.org/10.1016/j.heliyon.2018.e00601.
[22] T. Niikura, Y. Hashimoto, H. Tajima, M. Ishizaka, Y. Yamagishi, M. Kawasumi, M. Nawa, K. Terashita, S. Also, I. Nishimoto, A tripartite motif protein TRIM11 binds and destabilizes Humanin, a neuroprotective peptide against Alzheimer's disease-relevant insults, Eur. J. Neurosci. 17 (2003), https://doi.org/10.1046/j.1460-9568.2003.02553.x.
[23] A. Andreadis, Tau splicing and the intricacies of dementia, J. Cell. Physiol. 227 (2012) 1220–1225, https://doi.org/10.1002/jcp.22842.
[24] K. Takahashi, M. Ishida, H. Komano, H. Takahashi, SUMO-1 immunoreactivity co-localizes with phospho-Tau in APP transgenic mice but not in mutant Tau transgenic mice, Neurosci. Lett. 441 (2008) 90–93, https://doi.org/10.1016/j.neulet.2008.06.012.
[25] J.G. O'Rourke, J.R. Gareau, J. Ochaba, W. Song, T. Raskó, D. Reverter, J. Lee, A. M. Monteys, J. Pallos, L. Mee, M. Vashishtha, B.L. Apostol, T.P. Nicholson, K. Illes, Y.-Z. Zhu, M. Dasso, G.P. Bates, M. Difiglia, B. Davidson, E.E. Wanker, J.L. Marsh, C.D. Lima, J.S. Steffan, L.M. Thompson, SUMO-2 and PIAS1 modulate insoluble mutant huntingtin protein accumulation, Cell Rep. 4 (2013) 362–375, https://doi.org/10.1016/j.celrep.2013.06.034.
[26] S. Subramaniam, K.M. Sixt, R. Barrow, S.H. Snyder, Rhes, a striatal specific protein, mediates mutant-huntingtin cytotoxicity, Science 324 (2009) 1327–1330, https://doi.org/10.1126/science.1172871.
[27] T. Stankova, L. Piepkorn, T.A. Bayer, O. Jahn, M. Tirard, SUMO1-conjugation is altered during normal aging but not by increased amyloid burden, Aging Cell 17 (2018) e12760, https://doi.org/10.1111/acel.12760.
[28] E. Ficulle, M.D.S. Sufian, C. Tinelli, M. Corbo, M. Feligioni, Aging-related SUMOylation pattern in the cortex and blood plasma of wild type mice, Neurosci. Lett. 668 (2018) 48–54, https://doi.org/10.1016/j.neulet.2018.01.004.
[29] H. Takamura, Y. Nakayama, H. Ito, T. Katayama, P.E. Fraser, S. Matsuzaki, SUMO1 modification of tau in progressive supranuclear palsy, Mol. Neurobiol. 59 (2022) 4419–4435, https://doi.org/10.1007/s12035-022-02734-5.
[30] R. Rott, R. Szargel, V. Shani, H. Hamza, M. Savyon, F. Abd Elghani, R. Bandopadhyay, S. Engelender, SUMOylation and ubiquitination reciprocally regulate α-synuclein degradation and pathological aggregation, Proc. Natl. Acad. Sci. U.S.A. 114 (2017) 13176–13181, https://doi.org/10.1073/pnas.1704351114.
[31] J. Magalhaes, E. Tresse, P. Ejlerskov, E. Hu, Y. Liu, A. Marin, A. Montalant, L. Satriano, C.F. Rundsten, E.M.M. Carlsen, R. Rydbirk, A. Sharifi-Zarchi, J. B. Andersen, S. Aznar, T. Brudek, K. Khodosevich, M. Prinz, J.-F.M. Perrier, M. Sharma, T. Gasser, S. Issazadeh-Navikas, PIAS2-mediated blockade of IFN-β signaling: a basis for sporadic Parkinson disease dementia, Mol. Psychiatr. 26 (2021) 6083–6099, https://doi.org/10.1038/s41380-021-01207-w.
[32] H. Wada, D. Suzuki, T. Niikura, Regulation of ALS-associated SOD1 mutant SUMOylation and aggregation by SENP and PIAS family proteins, J. Mol. Neurosci. 70 (2020) 2007–2014, https://doi.org/10.1007/s12031-020-01604-w.
[33] T. Maruyama, H. Wada, Y. Abe, T. Niikura, Alteration of global protein SUMOylation in neurons and astrocytes in response to Alzheimer's disease-associated insults, Biochem. Biophys. Res. Commun. 500 (2018) 470–475, https://doi.org/10.1016/j.bbrc.2018.04.104.