Effects of amyloid-β on protein SUMOylation and levels of mitochondrial proteins in primary cortical neurons

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\textbf{A R T I C L E   I N F O}

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\textbf{A B S T R A C T}

Defining the molecular changes that underlie Alzheimer’s disease (AD) is an important question in neuroscience. Here, we examined changes in protein SUMOylation, and proteins involved in mitochondrial dynamics, in an in vitro model of AD induced by application of amyloid-β 1–42 (Aβ\textsubscript{42}) to cultured neurons. We observed Aβ\textsubscript{42}-induced decreases in global SUMOylation and in levels of the SUMO pathway enzymes SENP3, PIAS1/2, and SAE2. Aβ exposure also decreased levels of the mitochondrial fission proteins Drp1 and Mff and increased activation of caspase-3. To examine whether loss of SENP3 is cytoprotective we knocked down SENP3, which partially prevented the Aβ\textsubscript{42}-induced increase in caspase-3 activation. Together, these data support the hypothesis that altered SUMOylation may play a role in the mechanisms underlying AD.

1. Introduction

Alzheimer’s disease (AD) is the most common neurodegenerative brain disorder (Breijyeh and Karaman, 2020), with aggregation of extracellular amyloid-beta peptide (Aβ) as a main histopathological hallmark. This Aβ pathology is initiated decades before the onset of clinical symptoms (Bateman et al., 2012). Given that there is no cure for AD, a key objective is to identify and understand the molecular mechanisms underlying AD, in order to intervene and restore neuronal function. Small ubiquitin-like modifier (SUMO) proteins are ~10 kDa proteins that are covalently conjugated to lysine residues in target proteins to modify their function. SUMO1 and SUMO2/3 paralogues are expressed in the mammalian brain and, in addition to their physiological function, are implicated in many neuropathologies, including AD (Princ and Tavernarakis, 2020). Recent studies indicate that increased SUMOylation of specific substrates can be neuroprotective in response to cell stress (Guo and Henley, 2014).

An enzymatic cascade mediates SUMOylation, starting with SUMO activation by an ATP-dependent E1 enzyme, formed by a heterodimer of SAE1 (AOS1) and SAE2 (UBA2). The activated SUMO is conjugated by the E2 conjugating enzyme Ubc9, which in combination with SUMO E3 ligases (including the PIAS family proteins), mediates target recognition and conjugation of SUMO to the substrate (Chang and Yeh, 2020). This process can be reversed by SUMO-specific proteases, the best characterized of which are the SENP family of six cysteine proteases (Guo and Henley, 2014).

Global increases in SUMO2/3 conjugation are a cellular protective response to severe ischemic stress (Datwyler et al., 2011), mediated by stress-induced loss of the deSUMOylating enzyme SENP3 (Guo et al., 2013). A key target for SENP3-mediated deSUMOylation is the GTPase dynamin-related protein 1 (Drp1), which plays a major role in regulating mitochondrial morphology and integrity (Kraus et al., 2021).
Recruitment of Drp1 to mitochondria during cell stress causes fragmentation and cytochrome c (cyt c) release, potent signals of cell death (Chang and Blackstone, 2010), which can be attenuated by enhanced Drp1 SUMOylation resulting from loss of SENP3 (Guo et al., 2017). This suggests that changes in SENP3 stability could provide new drug targets and therapeutic strategies.

Here we investigated global SUMOylation, SUMO pathway enzymes, and changes in proteins relevant to mitochondrial dysfunction and neuronal death in an in vitro Aβ and changes in proteins relevant to mitochondrial dysfunction and Drp1 SUMOylation resulting from loss of SENP3 (Guo et al., 2017). This approach is based on the idea that loss of SENP3, an important regulator of mitochondrial dynamics, may promote cell survival in this in vitro AD model.

2. Material and methods

2.1. Cell culture

Cortical cultures were prepared from E18 Wistar rat embryos, using a modified protocol (Martin et al., 2007). All animal care and procedures were carried out in full compliance with University of Bristol and ARRIVE guidelines, and the UK Animals Scientific Procedures Act, 1986. In addition, all experimental protocols were approved by University of Bristol Animal Welfare and Ethics Review Body (ethics approval number UIN: UB/18/004) panel and the Biological and Genetic Modification Safety Committee (BGMSC).

Cells were plated on poly-L-lysine-coated culture dishes at a density of 600000 per well (6-well plates) in Neurobasal medium (Gibco) containing B27 and 2 mM glutamine and incubated at 37 °C. Twenty-four hours later, the media was replaced by Neurobasal medium containing B27 and 2 mM glutamine and incubated at 37 °C in humidified air supplemented with 5% CO2 until experimental usage.

2.2. Lentivirus production

Scrambled shRNA or shRNA targeting SENP3 (target sequence TATGGACAGACCTTGCTCAATGACAGGT) (Rawlings et al., 2019) were cloned into a modified pXGL3 lentiviral vector under the control of a U6 promoter. Lentivirus was produced in HEK293T cells using the helper vectors p8.91 and pMD2. G, as described previously (Rocca et al., 2017).

2.3. Aβ aggregation and application to cultures

Aβ1–42 or its reverse peptide Aβ42–1 (Bachem) were incubated in 0.2% ammonium hydroxide in Milli-Q water (1 mg/mL) at 37 °C for 4 days before use. Aliquots were stored at ~ −8 °C. Primary neuronal cultures were exposed to 2 µM Aβ1–42 or Aβ42–1 for 48 h, at 17 days in vitro (DIV) (Hoppe et al., 2013a,b). In some experiments, primary cortical neurons were infected with either scrambled shRNA or SENP3 shRNA lentivirus for 4 days prior to 2 µM Aβ1–42 or Aβ42–1 challenge.

2.4. Immunoblotting

Cells were lysed directly into 1x Laemml sample buffer, collected in eppendorfs and heated to 95 °C for 10 min prior to SDS-PAGE. Western blots were immunoblotted with the following antibodies: SUMO1 (Cell Signaling Technology, 1:1000), SUMO2/3 (Cell Signaling Technology, 1:1000), SENP3 (Cell Signaling Technology, 1:1000), Ubc9 (Cell Signaling Technology, 1:1000), PIAS1/2 (Abcam, 1:1000), UBA2 (Santa Cruz Biotechnology, 1:500), Drp1 (BD Biosciences, 1:2000), Mff (Santa Cruz Biotechnology, 1:500), Fis1 (ProteinTech, 1:1000), Mfn2 (Cell Signaling Technology, 1:2000), OPA1 (Abcam, 1:1000), AMPKα (Cell Signaling Technology, 1:1000), p-AMPKα (Cell Signaling Technology, 1:1000) and cleaved caspase-3 (Cell Signaling Technology, 1:1000). Horseradish peroxidase-conjugated anti-mouse and anti-rabbit IgG (Sigma, 1:10000) were used as secondary antibodies and GAPDH (Abcam, 1:20000) was used as loading control. Each immunoblot is representative of at least three experiments carried out using different cell populations and analysed using ImageJ software (NIH, USA).

2.5. Statistics

Statistical analyses were performed using Student’s t-test or two-way ANOVA followed by Fisher post-hoc test when appropriate. Statistical significance was considered at p ≤ 0.05. Values are presented as mean ± standard error of the mean (S.E.M.) and expressed as percentage of the control Aβ42–1 value.

3. Results

3.1. Aβ1–42 decreases SUMO1 and SUMO2/3 conjugation

We first analysed the effects of Aβ1–42 or its inactive reverse peptide Aβ42–1 (2 µM) on global SUMOylation in primary cortical neurons incubated with Aβ for 48 h. Exposure to Aβ1–42 caused a global decrease in both SUMO1 and SUMO2/3 conjugation to proteins (Fig. 1). Furthermore, analysis of a selection of prominent bands revealed significant changes in individual SUMO1 and SUMO2/3 conjugates (Table 1). Distinct bands on the SUMO1 blot showed decreases at ~80 kDa and ~45 kDa, while SUMO2/3 reactive bands at ~40 kDa and ~30 kDa were also decreased by Aβ1–42 (Table 1). Crucially, no differences were observed between cells treated with the reverse control peptide Aβ42–1 and untreated cells (Supplementary Tables 1, 2 and 3).

3.2. Aβ1–42 reduces levels of important enzymes involved in the SUMOylation pathway

We next evaluated the effects of Aβ1–42 on key enzymes in the SUMOylation pathway. Total levels of the SUMO2/3-selective protease SENP3 were dramatically reduced by Aβ1–42 (Fig. 2A), whereas levels of the only SUMO E2 conjugating enzyme, Ubc9, remained unchanged (Fig. 2B). Interestingly, however, levels of both the SUMO E3 ligases PIAS1/2 (Fig. 2C) and the SUMO activating enzyme SAE2 (Fig. 2D) were reduced by Aβ1–42.

3.3. Effects of Aβ1–42 on mitochondrial proteins

Mitochondria are highly dynamic organelles that constantly undergo repeated cycles of fission and fusion to maintain appropriate numbers and to mediate quality control (Hers et al., 2017). Mitochondrial fission involves the recruitment of the GTPase and SUMO substrate Drp1 from the cytosol to the mitochondrial surface via binding to adaptor proteins, one of which is mitochondrial fission factor (Mff) (Loson et al., 2013). Mitochondrial dysfunction or damage in neurons is closely associated with neurodegeneration (Bock and Tait, 2019), and altered mitochondrial dynamics have been observed in AD patients and models of AD (Wang et al., 2009), so we tested the effects of Aβ1–42 on the mitochondrial fission machinery. As shown in Fig. 3, Aβ1–42 reduces total levels of Drp1 and also leads to an increase in caspase-3 cleavage, a marker of apoptosis (Figs. 3A and 3B).

Mff is the dominant mitochondrial adaptor protein for Drp1 (Loson et al., 2013) and its levels are also decreased by Aβ1–42 (Fig. 3C). It has been proposed that another Drp1 adaptor protein, mitochondrial fission protein 1 (Fis1), plays an ancillary role, acting after Drp1 and Mff initiate fission (Shen et al., 2014). We found that Fis1 levels are unaltered by Aβ1–42 (Fig. 3D). We also investigated the levels of adenosine trifluoromethyl ketone ( SKF).
monophosphate (AMP)-activated protein kinase (AMPK), which is rapidly activated by mitochondrial stress and triggers mitochondrial fission, at least in part, via phosphorylation of Mff (Toyama et al., 2016).

Again, Aβ1–42 treatment did not change the levels of total AMPK or its active phosphorylated form (Fig. 3E).

Since we observed changes in levels of the fission GTPase Drp1 in response to Aβ1–42, we also tested whether levels of the fusion GTPases Mfn2, which is responsible for fusion at the mitochondrial outer membrane, or OPA1, which mediates inner membrane fusion (Mishra and Chan, 2014), were altered by Aβ1–42 application. However, neither Mfn2 nor OPA1 levels were altered by Aβ1–42 (Figs. 3F and 3G).

### Table 1

| SUMO1 conjugation | SUMO2/3 conjugation |
|-------------------|---------------------|
| High MW ~80 kDa   | ~45 kDa             |
| 100               | 100                 |
| 100               | 100                 |
| 100               | 100                 |
| 100               | 100                 |
| 100               | 100                 |
| 100               | 100                 |
| Aβ42-1            | 100                 |
| Aβ1-42            | 81.78 ± 17.3        |
|                   | 72.63 ± 10.9        |
|                   | 40.08 ± 10.1        |
|                   | 11.5 ± 1.5          |
|                   | 7.9 ± 0.9           |
|                   | 19.4 ± 2.4          |
|                   | 8.5 ± 0.5           |
|                   | 14.1 ± 1.1          |

Results are presented as mean ± S.E.M. and expressed as percentage of Aβ42-1 value. N = 4 for high MW SUMO1 conjugation and all SUMO2/3 analysis), N = 5 for the ~80 kDa SUMO1 band, and N = 6 for the ~45 kDa SUMO1 band. Statistical analyses were performed using Student’s t-test. *p < 0.05.

Aβ1-42: amyloid-beta peptide; MW: Molecular Weight; kDa: kilodaltons; S.E.M.: Standard Error of the Mean.

3.4. SENP3 depletion partially protects against the increase of cleaved caspase-3 induced by Aβ1-42

Previous studies have demonstrated that loss of SENP3 is neuroprotective against ischemic stress and heavy metal-induced toxicity (Guo et al., 2013; Guo et al., 2017). We therefore, wondered if loss of SENP3 could reverse the decrease in SUMOylation observed after application of Aβ1-42, and potentially promote cell survival. Consistent with this hypothesis, shRNA-mediated knockdown of SENP3 counteracted the decreased levels of protein SUMO2/3-ylation induced by Aβ1-42 (Fig. 4 A and 4B). Moreover, SENP3 knockdown partially rescued the Aβ1-42-induced increase in cleaved caspase-3 (Fig. 4 C), suggesting that loss of SENP3 may promote cell survival in this in vitro model of AD.
4. Discussion

Our hypothesis was that since SUMOylation is an important regulator of synaptic and neuronal function and dysfunction (Guo and Henley, 2014), it likely plays key roles in the neuronal deficits that lead to AD. Indeed, altered SUMOylation dynamics have been observed in both human AD brain, and in animal models of AD (McMillan et al., 2011). Furthermore, perturbed mitochondrial dynamics are a central factor in AD pathology (Lin and Beal, 2006), but the mechanisms and pathways involved are poorly characterized.

Here, we used an in vitro Aβ1–42 application model to examine changes in SUMOylation, SUMO pathway enzymes and essential mitochondrial proteins in AD. We used 2 µM Aβ1–42 that, while still a high concentration compared to the pathophysiological levels measured in in vivo transgenic mouse models (Maia et al., 2015), has been used previously in studies from our group and others (Hoppe et al., 2013a, 2013b; Tarczyluk et al., 2015; Wällti et al., 2018). Both SUMO1 and SUMO2/3 conjugation were decreased by 2 µM Aβ1–42. Consistent with this, the SUMO-activating enzyme SAE2 and the SUMO E3 ligase PIAS1/2 were reduced by Aβ1–42. Somewhat counterintuitively, given that total SUMO2/3 conjugation is reduced, the SUMO2/3-specific deSUMOylating enzyme SENP3 was also decreased by Aβ1–42. At first sight, this would lead to the prediction of increased SUMO2/3ylation. However, SUMOylation pathways are highly dynamic and complex, and the increase that may result from SENP3 loss may be offset by our observed decreases in the E1 and E3 enzymes. Furthermore, reliable antibodies are not yet available for several rat SUMO proteases, so we cannot currently exclude the possibility that reduced SENP3 may also be accompanied by increases in other SUMO proteases. Nonetheless, it has previously been reported that SENP3 expression is downregulated in microarray analyses of sporadic AD tissues (Weeraratna et al., 2007), and the brains of human Down Syndrome (DS) patients (Binda et al., 2017). Since amyloid precursor protein (APP) is encoded on the trisomic chromosome in DS, DS patients exhibit advanced amyloid pathology and early-onset dementia, further supporting our observed link between Aβ deposition and SENP3 levels.

Interestingly, a recent paper reported no significant changes in SUMO1 or SUMO2/3 conjugation in response to Aβ1–42 treatment of cultured cortical neurons, in contrast to the decrease we observed here (Maruyama et al., 2018). While the reasons for this discrepancy are currently unclear, this may be due to differences in the Aβ1–42 oligomerization protocol used. We looked at the effects of aggregated Aβ1–42 peptide preparations, which are predominantly fibrillar rather than soluble or oligomeric (Ferreira et al., 2015). Moreover, the decrease in conjugation observed here may be a result of the higher concentration of Aβ1–42 (2 µM versus 1 µM) and longer duration of insult (48 h versus 1 or 24 h) than that used by Maruyama et al. (2018), which likely results in a more severe model of AD-like stress.

Impaired mitochondrial dynamics have been reported to result in excessive mitochondrial fragmentation in AD (Wang et al., 2009). In particular, alterations in the GTPase Drp1 have been proposed to contribute to abnormal mitochondria function (Wang et al., 2009;
Fig. 3. Aβ1–42 decreases Drp1 (A) and Mff (C) levels and increases cleaved caspase-3 (B). Levels of Fis1 (D), AMPK, phospho-AMPK (E), Mfn2 (F) and OPA1 (G) were unchanged. DIV17 rat cortical neuronal cultures were incubated with 2 μM Aβ1–42 or its reverse peptide for 48 h. GAPDH was used as a loading control. N = 6 (Drp1, Mff, cleaved caspase-3, Fis1, phospho-AMPK and total AMPK) and N = 4 (Mfn2 and OPA1). *p ≤ 0.05, Student’s t-test, compared to Aβ42–1 group.
Here, we observed a decrease in Drp1 levels in response to Aβ42, while levels of the fusion GTases Mfn2 and OPA1 were unchanged. At first sight, these results may seem surprising, given previous reports of mitochondrial fragmentation resulting from excessive fission in AD (Shen et al., 2014; Baek et al., 2017). However, it is important to note that levels of fission are dependent on mitochondrial recruitment of Drp1 (Wang et al., 2009), and thus changes in total Drp1 levels may not entirely be reflected in changes in the active mitochondrial pool of Drp1. Indeed, a previous study examining the mitochondrial association of Drp1 in human AD brain samples observed that although total levels of Drp1 were decreased in AD, the mitochondrial pool of Drp1 was increased, resulting in a fragmented phenotype (Wang et al., 2009). While we observed significant changes in the expression levels of SUMOylation and mitochondrial proteins, specific Aβ42-induced changes in the levels of SUMOylated Drp1 remain to be demonstrated. Further work will therefore be required to determine how mitochondrial recruitment of Drp1, and post-translational modifications of Drp1 which control this process, such as SUMOylation (Guo et al., 2013; Guo et al., 2017), are altered by Aβ42 treatment.

Interestingly, and consistent with previous studies, we show that shRNA knockdown of SENP3 increased SUMO2/3ylation, and partially prevented Aβ42-induced caspase-3 cleavage. We have shown previously that SENP3 degradation during ischemia represents a cellular protective response and that knockdown of SENP3 before the insult promotes cell survival (Guo et al., 2013; Guo et al., 2017). Our current data suggest that SENP3 loss also occurs in AD models and that promoting SENP3 loss before AD-like stress favours cell survival. Further work will be required to determine exactly how SENP3 loss enhances cell survival in AD models. A likely possibility is that it functions, at least in part, by promoting SUMOylation of its substrate Drp1, to reduce excessive mitochondrial fission and resulting apoptosis.

Overall, our data demonstrate that Aβ42 decreases global SUMO1 and SUMO2/3 conjugation to target proteins in cultured neurons. Since SUMOylation is proposed to be neuroprotective, we hypothesize that this reduction in target protein SUMOylation could contribute to synaptic, mitochondrial and neuronal dysfunction in AD. Consistent with this concept, Aβ42 perturbed mitochondrial proteins and increased caspase-3 cleavage, a core component of the apoptotic pathway. While much more work remains to be done, our results indicate that protein SUMOylation, and SENP3 in particular, could represent a tractable target for beneficial manipulation to reduce neuronal dysfunction in AD.

4.1. Limitations of the study and future work

In our study, we have examined levels of protein SUMOylation, SUMOylation-associated proteins, and levels of mitochondrial proteins after application of Aβ42. Furthermore, we have investigated the potential for SENP3 knockdown to reduce the induction of cell death markers after Aβ42 treatment. While we believe these findings provide important information that will form the basis of future work by ourselves and others, we are mindful of a number of limitations of our study that should be considered when interpreting our findings.

We have used Western blotting to assess levels of our proteins of interest, and to measure levels of the established apoptotic marker cleaved caspase-3. However, we note that the veracity of these immunoblot data would be complemented by additional immunofluorescence studies. Additionally, it would be useful to perform additional cell viability assays to confirm that the activation of caspase-3 we observe upon Aβ42 application accurately reports cell death. Moreover, since mitochondrial recruitment of Drp1 dictates its function in fission, further studies examining colocalization of Drp1 with mitochondria will be informative in determining how the Aβ42-induced changes in total Drp1 levels observed here impact upon Drp1 recruitment to mitochondria, and how Aβ42 effects mitochondrial morphology more generally. Finally, we are aware our findings report the effects of one concentration and exposure time of fibrillar Aβ42 on cultured cortical neurons. Since Aβ42 concentrations, exposure times, oligomerisation strategies, and neuronal preparation protocols differ widely in the field, further work will also be required to determine whether our findings are universally reproduced in these various model systems. Notwithstanding these areas for future research, we content that our data provide compelling evidence for key roles of SUMOylation and SENP3-mediated deSUMOylation of mitochondrial proteins in the regulation of neuronal viability in a model of AD.

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Declaration of interest

None.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.brainres.2022.01.003.

References

Baek, S.H., Park, S.J., Jeong, J.I., Kim, S.H., Han, J., Kyung, J.W., Baik, S.H., Choi, Y., Choi, B.Y., Park, J.S., Bahn, G., Shin, J.H., Jo, D.S., Lee, J.Y., Jung, C.G., Arumugam, T.V., Kim, J., Han, J.W., Koh, J.Y., Jo, D.G., 2017. Inhibition of Drp1 ameliorates synaptic depression, abeta deposition, and cognitive impairment in an Alzheimer’s disease model. J. Neurosci. 37 (20), 5099-5110. https://doi.org/10.1523/jneurosci.2385-16.2017.

Bateman, R.J., Xiong, C., Benzinger, T.L., Fagan, A.M., Goate, A., Fox, N.C., Marcus, D.S., Datwyler, A.L., Lattig-Tunnemann, G., Yang, W., Paschen, W., Lee, S.L., Dirnagl, U., Bristol, UK) for excellent technical assistance.

Chang, C.R., Blackstone, C., 2010. Dynamic regulation of mitochondrial fission through modification of the dynamin-related protein Drp1. Annu. N. Y. Acad. Sci 1201, 34–39. https://doi.org/10.1111/j.1749-6632.2010.05629.x.

Chang, H.M., Yeh, E.T.H., 2020. SENP3-mediated desUMOylation of desUMOylation of Drp1 facilitates interaction with Mff to promote cell death following ischaemia. EMBO J. 32 (11), 1514–1528. https://doi.org/10.1093/embj.2013.65.

Guo, C., Wilkinson, K.A., Evans, A.J., Rubin, P.P., Henley, J.M., 2017. SENP3-mediated desUMOylation of Drp1 promotes interaction with Mff to promote cell death. Sci Rep. 7 (43811), 1–11. https://doi.org/10.1038/srep43811.

Hampel, H., Hardy, J., Bannen, K., Chen, C., Perry, G., Kim, S.H., Villemagne, V.L., Aisen, P., Vendruscolo, M., Iwayuato, T., Masters, C.L., Cho, M., Lannfelt, L., Cummings, J.L., Vergallo, A., 2021. The amyloid-β pathway in Alzheimer’s disease. Mol. Psychiatry 1–23. https://doi.org/10.1038/s41386-021-01249-0.

Herst, P.M., Rowe, M.R., Carson, G.M., Berridge, M.V., 2017. Functional mitochondria in health and disease. Front. Endocrinol. 8, 1–16. https://doi.org/10.3389/fendo.2017.00206.

Hoppe, J.B., Haag, M., Whalley, B.J., Salibego, C.G., Cimarosti, H., 2013a. Curcumin protects organotypic hippocampal slice cultures from Aβ1-42-induced synaptic toxicity. Toxicon. In Vitro 27 (8), 2325–2330. https://doi.org/10.1016/j.tiv.2013.02.002.

Hoppe, J.B., Rattray, M., Tu, H., Salibego, C.G., Cimarosti, H., 2013b. SUMO-1 conjugation blocks beta-amyloid-induced astrocyte reactivity. Neurosci. Lett. 546, 51–56. https://doi.org/10.1016/j.neulet.2013.04.050.

Kraus, F., Roy, K., Pucadyil, T.J., Ryan, M.T., 2021. Function and regulation of the divirome for mitochondrial fusion. Nature 590 (7844), 57–66. https://doi.org/10.1038/s41586-021-02314-x.

Lin, M.T., Beal, M.F., 2006. Alzheimer’s APP mangles mitochondria. Nat. Med. 12 (11), 1241–1245. https://doi.org/10.1093/nmcl/12.11.1241.

Loson, O.C., Song, Z., Chen, H., Chan, D.C., 2013. Fis1, Mff, MiD49, and MiD51 mediate Drp1 recruitment in mitochondrial fusion. Mol. Biol. Cell. 24 (5), 659–667. https://doi.org/10.1091/mbc.e12-09-0721.

Mai, I.F., Kaesser, S.A., Reichwald, J., Lambert, M., Obermüller, U., Schelle, J., Odenthal, J., Martus, P., Staufnhein, M., Jucker, M., 2015. Increased CSF Aβ during the very early phase of cerebral Aβ deposition in mouse models. EMBO Mol. Med. 7 (7), 895–903. https://doi.org/10.15252/emmm.201505029.

Martin, S., Nishimune, A., Meller, J.R., Henley, J.M., 2007. SUMOylation regulates kainate-receptor-mediated synaptic transmission. Nature 447 (7142), 321–325. https://doi.org/10.1038/nature05736.

Maruyama, T., Wada, H., Abe, Y., Niikura, T., 2018. Alteration of global protein SUMOylation in neurons and astrocytes in response to Alzheimer’s disease-associated insults. Biochem. Biophys. Res. Commun. 500 (2), 470–475. https://doi.org/10.1016/j.bbrc.2018.04.104.

McMillan, L.E., Brown, J.T., Henley, J.M., Cimarosti, H., 2011. Profiles of SUMO and ubiquitin conjugation in an Alzheimer’s disease model. Neurosci. Lett. 502 (3), 201–208. https://doi.org/10.1016/j.neulet.2011.07.045.

Mishra, P., Chan, D.C., 2014. Mitochondrial dynamics and inheritance during cell division, development and disease. Nat. Rev. Mol. Cell Biol. 15 (10), 634–646. https://doi.org/10.1038/nrm3877.

Prince, A., Tavnerarakis, N., 2020. SUMOylation in Neurodegenerative Diseases. Gerontology 66 (2), 122–130. https://doi.org/10.1159/000502142.

Rawlings, N., Lee, L., Nakamura, Y., Wilkinson, K.A., Henley, J.M., 2019. Protective role of the deSUMOylating enzyme SENP3 in myocardial ischemia-reperfusion injury. PloS one 14 (4), 1–17. https://doi.org/10.1371/journal.pone.0213311.

Rocca, D.L., Wilkinson, K.A., Henley, J.M., 2017. SUMOylation of FOXO1 regulates transcriptional repression via CBP1 to drive dentritic morphogenesis. Sci. Rep 7 (877), 1–12. https://doi.org/10.1038/s41598-017-00797-4.

Shen, Q., Yamano, K., Head, B.P., Kawajiri, S., Chung, J.T., Wang, C., Cho, J.H., Hattori, N., Youle, R.J., van der Bliek, A.M., 2014. Mutations in Fis1 disrupt orderly neuroprotective mechanism. J. Cereb. Blood Flow Metab. 34 (11), 2152–2159. https://doi.org/10.1038/jcbfm.2014.1112.

Ferreira, S.T., Lourenço, M.V., Oliveira, M.M., De Felice, F.G., 2015. Soluble amyloid-β oligomers as synaptotoxins leading to cognitive impairment in Alzheimer’s disease. Front. Cell. Neurosci 9 (191), 1–17. https://doi.org/10.3389/fncel.2015.00191.

Guo, C., Wilkinson, K.A., Evans, A.J., Rubin, P.P., Henley, J.M., 2017. SENP3-mediated desUMOylation of desUMOylation of Drp1 promotes interaction with Mff in response to energy stress. Science 351 (6270), 275–281. https://doi.org/10.1126/science.aab4138.

Walti, M.A., Steiner, J., Meng, F., Louis, H.S., Liu, J.M., Ghrilanti, R., Tagiryan, V., Nath, A., Clore, G.M., 2018. Probing the mechanism of inhibition of amyloid-beta(1-42)-induced neurotoxicity by the chemokine CCR5. Nat. Protoc. Nat. Acad. Sci. U. S. A. 11 (51), E1924–E1930. https://doi.org/10.1038/nprot.2018.057.

Wang, X., Su, B., Lee, H.G., Li, X., Perry, G., Smith, M.A., Zhu, X., 2009. Impaired balance of mitochondrial fusion and fission in Alzheimer’s disease. J. Neurosci. 29 (28), 9090–9093. https://doi.org/10.1523/jneurosci.1357-09.2009.

Weeraratana, A.T., Kalehua, A., Deleon, I., Bertak, D., Maher, G., Wade, M.S., Lustig, A., Becker, K.G., Wood 3rd, W., Walker, D.G., Beach, T.G., Taub, D.D., 2007. Alterations in immunological and neurological gene expression patterns in Alzheimer’s disease tissues. Exp. Cell. Res. 313 (3), 450–461. https://doi.org/10.1016/j.yexcr.2006.10.028.