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Targeting endoplasmic reticulum acetylation to restore proteostasis in Alzheimer's disease

This scientific commentary refers to 'Improved proteostasis in the secretory pathway rescues Alzheimer's disease in the mouse', by Peng *et al.* (doi:10.1093/brain/awv385).

Maintaining the health of the proteome is essential for sustaining biological functions. The buffering capacity of the proteostasis network is reduced during ageing, which represents the major risk factor for most common neurodegenerative diseases. In fact, independent of the aetiology of the disease, the misfolding and aggregation of specific proteins is a hallmark of many neurodegenerative conditions, which are now classified as protein misfolding disorders. Quality control pathways recognize aberrant proteins and promote their clearance by different routes, in particular the ubiquitin–proteasome system and macroautophagy (hereafter referred to as autophagy) (Vilchez *et al.*, 2014). The endoplasmic reticulum (ER) is the subcellular compartment responsible for protein synthesis and folding of nearly one-third of the total proteome. Several homeostatic mechanisms control the fidelity and efficiency of the protein folding process at the ER, including the unfolded protein response (UPR), the ER-associated degradation (ERAD) pathway, and the calnexin and calreticulin cycle, among others. Recently, new post-translational modifications of ER clients were discovered in the form of acetylation of lysines, an event that serves as quality control of protein-folding intermediaries. In this issue of *Brain*, Peng and co-workers report that inhibiting the acetylation of nascent proteins can control ER proteostasis through a novel mechanism that modulates autophagy, providing neuroprotection in models of Alzheimer's disease (Peng *et al.*, 2016).

Maintaining the efficiency of the protein-folding process in the ER represents a constant challenge for the cell, where proteins with several hydrophobic transmembrane domains are folded with low rates of success. Furthermore, most secretory proteins undergo sequential post-translational modifications including glycosylation, disulfide bond formation, glycophosphatidylinositol (GPI) tagging, and proteolytic processing, in addition to the assembly of multimeric protein complexes. A dynamic network of ER factors assists the process of protein folding to minimize the accumulation of toxic and unstable folding species that are highly prone to aggregation (Schroder and Kaufman, 2005). ERAD is the major pathway by which misfolded or unfolded proteins accumulated in the ER are retro-translocated to the cytosol for proteasome-mediated degradation. The proteasome preferentially degrades monomeric proteins that require unfolding prior to retro-translocation across the ER membrane, whereas autophagy favours the clearance of proteins in an aggregated state (Vilchez *et al.*, 2014). Autophagy is a catabolic process that allows the recycling of cellular components, which are initially engulfed into double-membrane phagophores that then fuse with lysosomes for cargo degradation. Autophagy also allows the disposal of misfolded proteins via their transfer from the ER to the lysosome through poorly described mechanisms, a process termed ERAD-II (Vilchez *et al.*, 2014). Importantly, the impairment of protein degradation pathways is emerging as a driving factor in protein misfolding disorders, whereas strategies to engage autophagy are protective in certain disease conditions (Vidal *et al.*, 2014).

Nε-lysine acetylation of proteins in the lumen of the ER was discovered in 2007 as a factor regulating the biosynthesis of BACE1, the beta secretase that processes amyloid precursor protein (Costantini *et al.*, 2007). Subsequent proteomic studies have assessed the 'ER acetylome' and predicted wide-ranging biological implications of this pathway (Pehar and Puglielli, 2013). ER acetylation is a reversible process mediated by a series of enzymes, including AT-1, a membrane transporter that translocates acetyl-CoA from the cytosol to the ER lumen, and ATase1 and ATase2, two acetyltransferases that modify ER cargo proteins (Pehar and Puglielli, 2013). The acetylation pathway may be dynamically regulated by ER stress since the *AT-1* gene is a target of the unfolded protein

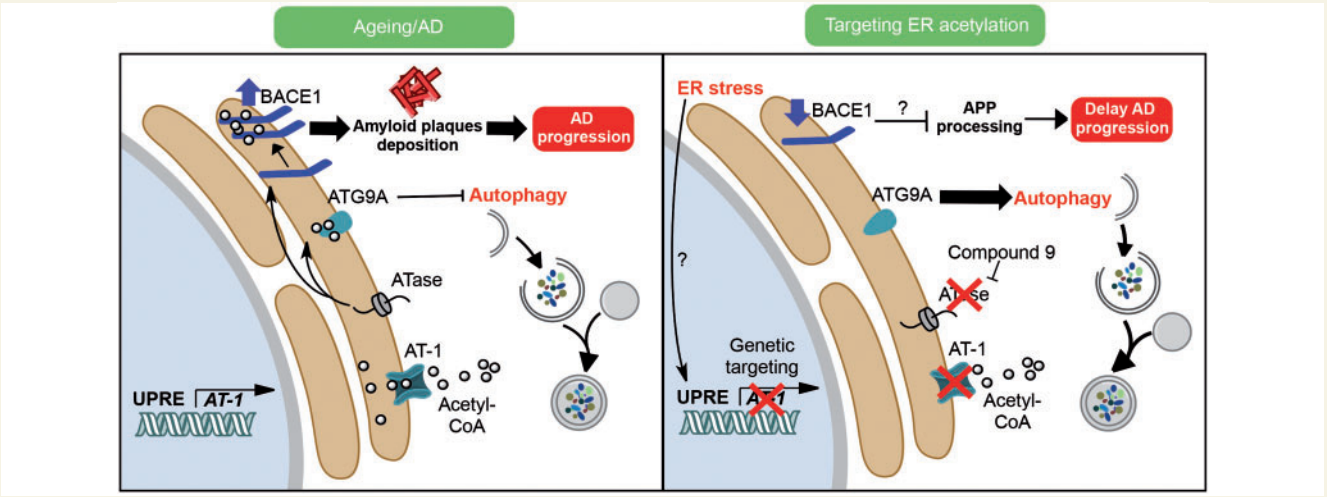

Figure 1 ER acetylation, autophagy and protein aggregation. Nε-lysine acetylation occurs transiently in the ER. ER acetylation is mediated by a series of enzymes, including AT-1 (transporter of acetyl-CoA), and the acetyltransferases ATase1 and ATase2, which can be inhibited by Compound 9. The ER acetylation pathway is engaged during ER stress through transcriptional control of a UPR element (UPRE). ER acetylation of proteins such as ATG9A may negatively regulate autophagy. Genetic inactivation of AT-1 triggers ER stress and autophagy. In the context of Alzheimer's disease (AD), targeting AT-1 reduces α-secretase BACE1 levels, a direct target of ER acetylation, and reduces amyloid precursor protein (APP) processing and amyloid-β levels, offering protection against the disease.

response. Recently, ER acetylation was genetically modified *in vivo* (Peng *et al.*, 2014). Targeting *At-1* function in mice leads to the appearance of neurodegenerative features and inflammation that correlate with enhanced autophagy (Peng *et al.*, 2014). At the mechanistic level, ER acetylation of autophagy-related proteins such as ATG9A may operate as a negative signal regulating this pathway.

Here, Peng and co-workers demonstrate that manipulation of AT-1 using S113R mutant cells that are devoid of acetyl-CoA transport activity enhanced the delivery of misfolded proteins to the autophagy compartment. Unexpectedly, ER acetylation specifically affected the disposal of misfolded proteins that were formed within the secretory pathway but not the cytosol (Fig. 1). Taking advantage of the existence of a knock-in AT-1^{S113R} heterozygous mouse, the authors performed extensive *in vivo* studies to define the contribution of ER acetylation to various neurodegenerative conditions. Consistent with their prediction, AT-$1^{S113R/+}$ mice were protected against Alzheimer's disease, but not Huntington's disease or amyotrophic lateral sclerosis, possibly due to the fact that the protein aggregates triggering neurodegeneration in the latter disease models (mutant Huntingtin and SOD1, respectively) preferentially accumulate in the cytosol (Peng *et al.*, 2016). Interestingly, targeting AT-1 *in vivo* also upregulated UPR markers, suggesting global effects on the ER proteostasis network.

The clinical features of Alzheimer's disease are associated with the presence of amyloid plaques and neurofibrillary tangles, which are assembled through extracellular deposition of misfolded amyloid-β peptide and intracellular hyper-phosphorylated tau, respectively. AT-$1^{S113R/+}$ mice were protected against experimental Alzheimer's disease, with increased synaptic plasticity, decreased load of soluble amyloid-β, reduced levels of BACE1 and improved survival. To probe the therapeutic potential of ER acetylation in Alzheimer's disease, Peng and co-workers tested a pharmacological strategy to manipulate the pathway. Using Compound 9, a small molecule that inhibits the acetyltransferases ATase-1 and ATase-2, the authors performed proof-of-concept experiments by treating Alzheimer's disease mice over an extended period as the animals aged. Importantly, Compound 9 reached the brain after systemic administration with no overall toxicity. Remarkably, administration of Compound 9 attenuated Alzheimer's disease features including the accumulation of hyperphosphorylated tau and soluble amyloid-β, in addition to increasing autophagy levels in the brain. Thus, the authors uncovered a selective and druggable ER-quality control mechanism with relevance to Alzheimer's disease.

Although all evidence to date links autophagy with neuroprotection after inhibition of ER acetylation, we speculate that there are three main mechanisms involved: (i) reducing BACE1 levels and APP processing; and (ii) rescuing specific autophagy defects in Alzheimer's disease (Lee *et al.*, 2010). Alternatively, (iii) a hormesis mechanism of protection (Hetz and Mollereau, 2014) may partly explain the phenotypes described here. Inhibition of ER acetylation may trigger the accumulation of immature proteins inside the ER lumen, triggering mild and non-toxic ER stress as reported here, which could operate as an adaptive signal to trigger autophagy. Similar observations were reported in models of amyotrophic lateral sclerosis and Huntington's disease, where targeting

Glossary

Autophagy: Self-degradative process with functions including the removal of misfolded or aggregated proteins, and also damaged organelles.
ER stress: A cellular condition generated when misfolded proteins accumulate inside the endoplasmic reticulum.
Hormesis: Phenomenon whereby an agent that is toxic to a biological system at high doses has beneficial effects on that system at lower doses.
Proteostasis: Term derived from the words 'protein' and 'homeostasis'. Refers to the network of pathways by which cells control the synthesis, trafficking and degradation of proteins.

an essential component of the unfolded protein response shifts the proteostasis network towards induction of autophagy (Hetz *et al.*, 2009), providing neuroprotection; a crosstalk that may also depend on ER acetylation of proteins (Pehar and Puglielli, 2013).

The Alzheimer's disease neuropathological cascade begins many years before clinical onset with general alterations in protein homeostasis involving a slow deposition of misfolded proteins. Thus, strategies to remove toxic protein aggregates are becoming an attractive target for future therapeutic intervention in Alzheimer's disease and most PMDs (Vidal *et al.*, 2014). In summary, Peng and co-workers have uncovered a previously unanticipated post-translational regulation in the ER lumen that directly affects levels of Alzheimer's disease-related proteins, in addition to improving the disposal of toxic aggregates, and possibly damaged organelles, through their transfer to the autophagy compartment. Overall, the current study supports the concept of ER acetylation as a novel target to alleviate neurodegeneration. Defining the ER acetylome in the nervous system may shed light on the involvement of the pathway in other human diseases affecting ER client proteins.

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