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Alterations of protein homeostasis in Alzheimer's disease: beyond Procrustean bed of endoplasmic reticulum stress and unfolded protein response

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Alzheimer's disease (AD) is a major age-related form of dementia with a number of cases exponentially growing, causing enormous social and economic impact on individuals and society. Neuropathological hallmarks of AD, evident in postmortem AD brains, include a massive loss of the grey matter in the neocortex, extracellular deposition of amyloid-β (Aβ) in the form of senile plaques and cerebrovascular amyloid angiopathy, and intra-neuronal accumulation of neurofibrillary tangles, formed by hyper-phosphorylated tau protein. The (most popular) Aβ cascade hypothesis posits the causal role of the aberrant processing of amyloid precursor protein, leading to the release and accumulation of Aβ. This hypothesis stems (possibly erroneously) from the presumed similarity of sporadic AD to inherited, rare familial AD form, triggered by mutations in amyloid precursor protein itself and presenilins 1 and 2 that form a catalytic core of the amyloid precursor protein processing protease γ-secretase. For a long time Aβ cascade hypothesis guided drug development studies and clinical trials in AD field. However, the failure of clinical trials of potential anti-AD drugs reflects a much higher complexity of AD pathogenesis. The main conceptual achievement of the last three decades in AD research has been the understanding that the cellular and biochemical abnormalities precede, by several decades, the emergence of clinical symptoms, indicating that the onset of AD occurs at the youth/middle age of potential AD patients (Selkoe and Hardy, 2016). This highlights the preclinical and prodromal stages as the major window of opportunity for disease-modifying therapy (**Figure 1**).

Although accumulation of Aβ is universally acknowledged to trigger AD, a growing body of evidence suggests that Aβ-independent mechanisms, in particular, aberrant Ca^{2+} homeostasis alter cell proteostasis. Abnormalities of the latter seem to be one of the earliest events in AD pathogenesis (De Strooper and Karran, 2016; Selkoe and Hardy, 2016). Protein folding is among the most active and resource-consuming cellular activities. Robust quality control mechanisms oversee protein processing pathways. Even in a physiological context, about 15% of proteins in the endoplasmic reticulum (ER) fail to fold correctly and undergo either refolding/reglycosylation or are subjected to a constitutive ER-associated degradation. When the accumulation rate of unfolded/misfolded/aggregated proteins exceeds the ER protein homeostatic capacity, misfolded proteins accumulate in the ER lumen, thus clogging the ER folding machinery and instigating ER stress. To resolve ER stress, a stereotypic 'unfolded protein response' (UPR) is procured. The UPR involves three canonical ER stress sensors, PRKR-like endoplasmic reticulum kinase, inositol-requiring enzyme 1, and activating transcription factor 6, activation of which suppresses global protein synthesis, while, concomitantly, activating transcription and synthesis of a set of proteins, involved in protein processing and stress response. The resolution of ER stress, as a result of adaptive UPR, leads to the normalization of proteostasis and cell survival, while failure to resolve ER stress initiates apoptotic cascade (called also apoptotic or terminal UPR) and cell elimination. Canonical ER stress/UPR is an evolutionarily conserved reaction to different endogenous and exogenous stressors (Hetz et al., 2020).

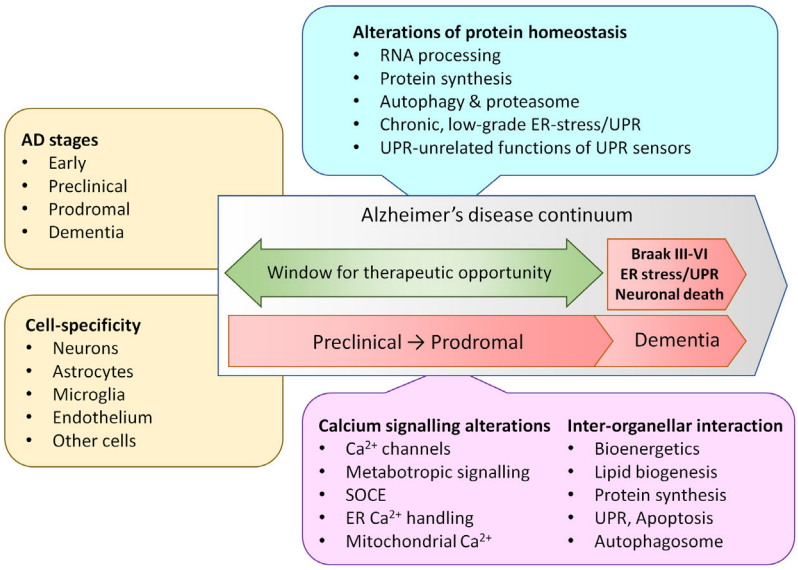

Figure 1 | **Factors that define the progression of AD pathogenesis.**
General factors include AD stages and brain region- and cell-specificity. Categories of specific factors, developing on its own yet interacting trajectories, include alterations of protein homeostasis, dysregulation of Ca^{2+} homeostasis, and inter-organellar interaction. Created using Microsoft PowerPoint. AD: Alzheimer's disease; ER: endoplasmic reticulum; SOCE: store-operated Ca^{2+} entry; UPR: unfolded protein response.

Because of the "proteinopathic" nature of AD, the concept and the mechanisms of canonical ER stress/UPR were widely exploited to understand the disease pathophysiology. Drugs targeting ER stress/UPR have been proposed for anti-AD therapy. This therapeutic strategy is supported by numerous experiments on primary neural cultures or cell lines, in which ER stress/UPR can be readily induced by exogenous application of rather high doses of Aβ, the latter acting similarly to classical ER stress instigators such as thapsigargin (inhibitor of SERCA pump) or tunicamycin (inhibitor of N-linked glycosylation). Likewise, direct delivery of Aβ into the rodent's brain induced robust ER stress with associated mobilization of UPR. In genetic animal AD models, however, contradictory data were produced. The validity of mice overexpressing AD-related mutant proteins has been questioned, when compared with knock-in models, expressing physiological protein levels (Hashimoto and Saido, 2018). Furthermore, analysis of human post-mortem material suggests that full activation of ER stress/UPR in AD brains emerges from Braak stage III onwards, in concomitance with the acceleration and spreading of tau pathology, and reaches full expression at Braak stages V–VI, corresponding to the profound neuronal death, clinically manifested by dementia (Lim et al., 2023). Altogether, this suggests that the execution of full-blown, terminal UPR leading to neuronal death, occurs in late AD.

These data indicate that the exploitation of the canonical form of ER stress/UPR for the development of a disease-modifying therapy may be misleading. Complex and somewhat paradoxical involvement of UPR with differential roles for distinct UPR ramifications in AD pathogenesis has been acknowledged and recently discussed (Gerakis and Hetz, 2018; Hetz et al., 2020). In part, activation of selected UPR subroutines may explain the UPR-unrelated role of canonical ER stress/UPR components. Growing number of reports suggests that canonical ER stress sensors and UPR transducers play different UPR-unrelated roles in cell physiology, in particular through their localization in specific sub-cellular structures such as inter-organellar contact sites, or interaction with cytoskeletal elements and regulation of distinct signaling pathways (Gerakis and Hetz, 2018; Hetz et al., 2020; Lim et al., 2023). Moreover, potentiation of selected UPR programs such as X-box binding protein 1, proved to be beneficial in AD animal models (Duran-Aniotz et al., 2023), Therefore, the activation of some components of canonical UPR does not necessarily signify full UPR activation or is harmful.

It is not clear why continuously increasing proteostatic stress, protracted for several decades from the very beginning of AD progression does not result in activation of terminal UPR and death of cells in the central nervous system until late AD and what the mechanisms allow cells to compensate, at the functional level, pathological changes. Below we discuss several aspects of AD pathophysiology, which, in our opinion, may significantly impact the interpretation of proteostatic alterations in general and ER stress/UPR in particular.

Chronic dysproteostasis and ER stress/UPR in pathogenesis of AD: Different types of ER stress/UPR were described. In cells that secrete high amounts of proteins, such as pancreatic β-cells, overloading the ER with proteins and constitutive UPR is physiological. Another variant of chronic ER stress/UPR is postulated for some cancer cells, which, in conditions of hypoxia and glucose shortage, undergo a transformation and repress apoptotic signaling in favor of survival and proliferation. In long-lasting progression of AD, increasing abnormalities of proteostasis gradually mount, in parallel with other ongoing pathological processes, such as, for example, dysregulation of Ca^{2+} homeostasis and mitochondrial decline, together creating a complex environment in which many small alterations interact with each other to produce a chronic cell malfunction and ultimate death. Slow accumulation of Aβ and/or other misfolded/unfolded proteins may not initially reach a hypothetic threshold for full UPR activation, or the activation might be gradual only for selected parts of UPR signaling. Nonetheless, such an environment is dynamic and evolves with the progression of the disease. Importantly, in spite of multiple functional and biochemical abnormalities, no overt neuronal death is observed until the late stages of AD (De Strooper and Karran, 2016; Lim et al., 2023). To explain the low level and/or inconsistent induction of ER stress/UPR, the terms "chronic" or "low-grade" ER stress/UPR are frequently used, but remain poorly defined (Dematteis et al., 2020; **Figure 1**).

Cell- and brain region-specificity of proteostatic alterations: Central nervous system is composed of different cell types including neurons, astroglia, microglia, oligodendroglia, and cells of blood vessels that form the active milieu of the nervous tissue (Semyanov and Verkhratsky, 2021). The ER stress and UPR were mostly studied in neurons. When whole brain tissue samples are examined, results are often (mis)interpreted through the lens of the neuronal alterations, overlooking the impact of other cell types. During terminal AD, while the number of neurons decreases substantially, the number of glial cells, specifically astrocytes, does not change significantly. In some models, astrocytes initiate ER stress/UPR. In human AD brains, astrocytic binding immunoglobulin protein/glucose-regulated protein 78 and C/EBP-homologous protein are co-expressed with glial fibrillary acidic protein and are upregulated in Braak VI but not in Braak 0–II stages (Lim et al., 2023). However, glial fibrillary acidic protein-positive reactive astrocytes are concentrated around senile plaques, while in plaque-free parenchyma astrocytes are either unchanged or atrophic. It is clear, therefore, that cell-specific alterations need to be investigated and have to be considered during the interpretation of data on whole tissue preparations (**Figure 1**).

A complex role of Ca^{2+} signaling: Calcium hypothesis of aging and dementia regards aging and age-related neurodegeneration as chronic calciumopathy (Verkhratsky and Toescu, 1998). In full compliance with the AD pathogenesis, the calcium hypothesis postulates that small alterations in Ca^{2+} signaling, accumulating throughout life, result in cell malfunction and, in the most extreme cases, cell death. Cellular Ca^{2+} signaling is a ubiquitous and versatile system coordinating cellular activities, hence its dysregulation contributes to many cell pathologies. Dysregulation of Ca^{2+} signaling has traditionally been considered detrimental. Terms such as "disruption" or "loss" of Ca^{2+} homeostasis or signaling are often used in the literature to label the differences between experimental conditions and control, without distinction of a specific pathway or functional outcome of the alteration. However, Ca^{2+} signaling is an umbrella term for many signaling events and pathways including homeostasis of Ca^{2+} ions in the cytosol and organelles, different Ca^{2+} fluxes, complex spatiotemporal patterns of Ca^{2+} signals as well as their decoding and transduction, all organized in a cell- and sub-cell-specific manner (Lim et al., 2021b).

Some "dysregulated" forms of Ca^{2+} signaling might dampen activation of ER stress/UPR and execution of cell death being, therefore, beneficial for cell survival and function. An example of such a favorable effect is represented by the cytosolic Ca^{2+} overload, increased store-operated Ca^{2+} entry, and ER Ca^{2+} dyshomeostasis. Increased resting cytosolic $[Ca^{2+}]$ as well as augmented store-operated Ca^{2+} entry may increase ER Ca^{2+} content, thus facilitating protein folding in the ER, counterbalancing ER stress. Another phenomenon, currently gaining attention is the role of the interaction between ER and mitochondria at the ER-mitochondria contact sites (Lim et al., 2021a). ER-mitochondria contact sites are a morpho-functional platform involved, among other functions, in the coordination of Ca^{2+} flux through inositol-1,4,5-trisphosphate receptor (InsP3R) and porin/voltage-dependent cation channel 1 (VDAC1). Direct Ca^{2+} transfer from the ER to mitochondrial inter-membrane space through the $InsP_3R$-VDAC1 complex drives a low-affinity mitochondrial Ca^{2+} uptake by mitochondrial Ca^{2+} uniporter supporting Ca^{2+}-dependent reactions in the mitochondrial matrix. In AD, extended interaction and shortening of the distance between ER and mitochondria impair Ca^{2+} flux from the ER to mitochondria, producing a dual effect: compromising mitochondrial bioenergetics and alleviating Ca^{2+}-dependent apoptosis (Dematteis et al., 2020; Lim et al., 2021a, 2023). Following this logic, a paradoxical conclusion can be drawn that the dysregulation of some Ca^{2+} signaling cascades during aging and AD exerts a protective effect being a kind of a "safety valve", suppressing full activation of ER stress/UPR and converting it into chronic/low-grade phenomenon, thus promoting functional compensation for several decades before the onset of symptoms (**Figure 1**).

Final remark: Aberrant proteostasis and Ca^{2+} dyshomeostasis are the prominent contributors to AD pathophysiology. Complexity of each of these phenomena requires a detailed investigation of the differential impact of selected components and molecular cascades in the context of their functional interaction. New tools and models need to be developed to address these questions at an adequate translational level.

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