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Autophagy Pathways, Ubiquitin-Proteasome System and Neurodegenerative Diseases: a Scopus Review

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Abstract. The maintenance of protein homeostasis is essential for neuronal health, and the failure of these mechanisms leads to the accumulation of misfolded proteins, such as α-synuclein, tau and Aβ, which form toxic aggregates, associated with diseases such as Alzheimer's, Parkinson's, Amyotrophic Lateral Sclerosis (ALS) and Huntington's. Protein balance is maintained by a complex network of molecular mechanisms, including chaperones, ubiquitin- proteasome system, autophagy, cellular stress response pathways that ensure the correct folding, degradation and elimination of defective proteins. However, factors such as mutations, environmental and metabolic stress can disrupt this network, in addition to aging, which reduces its effectiveness and performance, resulting in the formation of toxic protein aggregates. This systematic review reinforces the relevance of proteostasis in neuronal health and in the development of neurodegenerative diseases, suggesting that precise modulation of these pathways may be an effective therapeutic approach to slow the progression of these conditions. The work developed is an exploratory study, carried out through a bibliographic research. A literature search was carried out in the Medline, Elsevier, Lilacs and Capes databases of periodicals, published between 2014 and 2024. The selected studies underwent an evaluation of the eligibility criteria. The mechanisms of a proteostasis network were presented in detail and the understanding of these processes reveals new possibilities for treatment of neurodegenerative diseases, opening new perspectives for therapeutic interventions aimed at preserving proteostasis and, consequently, preventing these diseases.

Keywords: Proteostasis. Autophagy. Ubiquitin-proteasome System. Neurodegeneration.

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Vias de Autofagia, Sistema Ubiquitina-Proteassoma e Doenças Neurodegenerativas: uma Revisão Scopus

Resumo. A manutenção da homeostase proteica é essencial para a saúde neuronal, e a falha desses mecanismos leva ao acúmulo de proteínas mal dobradas, como α-sinucleína, tau e Aβ, que formam agregados tóxicos, associados a doenças como Alzheimer, Parkinson, Esclerose Lateral Amiotrófica (ELA) e Huntington. O equilíbrio proteico é mantido por uma rede complexa de mecanismos moleculares, incluindo chaperonas, sistema ubiquitina-proteassoma, autofagia, vias de resposta ao estresse celular que garantem o correto dobramento, degradação e eliminação de proteínas defeituosas. Porém, fatores como mutações, estresse ambiental e metabólico podem perturbar essa rede, além do envelhecimento, que reduz a eficácia e o rendimento, resultando na formação de agregados proteicos tóxicos. Esta revisão sistemática reforça a relevância da proteostase na saúde neuronal e no desenvolvimento de doenças neurodegenerativas, sugerindo que a modulação precisa destas vias pode ser uma abordagem terapêutica eficaz para retardar a progressão destas condições. O trabalho desenvolvido é um estudo exploratório, realizado por meio de pesquisa bibliográfica. Foi realizada pesquisa bibliográfica nos periódicos Medline, Elsevier, Lilacs e Capes, publicados de 2014 a 2024. Os estudos selecionados passaram por avaliação dos critérios de elegibilidade. Os mecanismos de uma rede de proteostase foram apresentados detalhadamente e a compreensão desses processos abre novas possibilidades de tratamento de doenças neurodegenerativas, abrindo novas perspectivas para intervenções terapêuticas visando a preservação da proteostase e, consequentemente, a prevenção dessas doenças.

Palavras-chave: Proteostase. Autofagia. Sistema Ubiquitina-Proteassoma. Neurodegeneração.

Vías de Autofagia, Sistema Ubiquitina-Proteasoma y Enfermedades Neurodegenerativas: una Revisión de Scopus

Resumen. El mantenimiento de la homeostasis de las proteínas es esencial para la salud neuronal, y el fallo de estos mecanismos conduce a la acumulación de proteínas mal plegadas, como la α-sinucleína, tau y Aβ, que forman agregados tóxicos, asociados con enfermedades como el Alzheimer, el Parkinson, la esclerosis lateral amiotrófica (ELA) y la enfermedad de Huntington. El equilibrio proteico se mantiene mediante una compleja red de mecanismos moleculares, que incluyen chaperonas, sistema ubiquitina-proteosoma, autofagia y vías de respuesta al estrés celular que aseguran el correcto plegamiento, degradación y eliminación de proteínas defectuosas. Sin embargo, factores como las mutaciones, el estrés ambiental y metabólico pueden alterar esta red, además del envejecimiento, que reduce la efectividad y el rendimiento, lo que resulta en la formación de agregados proteicos tóxicos. Esta revisión sistemática refuerza la relevancia de la proteostasis en la salud neuronal y el desarrollo de enfermedades neurodegenerativas, lo que sugiere que la modulación precisa de estas vías puede ser un enfoque terapéutico eficaz para frenar la progresión de estas enfermedades. El trabajo desarrollado es un estudio exploratorio, realizado a través de una investigación bibliográfica. Se realizó una búsqueda bibliográfica en las revistas Medline, Elsevier, Lilacs y Capes, publicadas entre 2014 y 2024. Los estudios seleccionados fueron sometidos a evaluación de los criterios de elegibilidad. Los mecanismos de una red de proteostasis se han presentado en detalle y la comprensión de estos procesos abre nuevas posibilidades para el tratamiento de enfermedades neurodegenerativas, abriendo nuevas perspectivas para intervenciones terapéuticas dirigidas a la preservación de la proteostasis y, en consecuencia, a la prevención de estas enfermedades.

Palabras clave: Proteostasis. Autofagia. Sistema Ubiquitina-Proteosoma. Neurodegeneración.

INTRODUCTION

Cellular proteostasis, or protein homeostasis, is related to the regulation of the proteome by controlling the concentration, conformation, binding interactions (quaternary structure), and localization of individual proteins, readapting functionality according to the needs of cells, often through transcriptional and translational changes. Frequent maintenance of proteostasis is of fundamental importance, ensuring normal and healthy cell development, as well as healthy aging and a robust response to the stress of environmental factors, including pathogen attacks, preventing the onset of a disease (Balch et al., 2008).

Protein quality control is one of the processes that influence proteostasis and serves as the main defense mechanism of cells against proteotoxicity induced by proteasome dysfunction (Zhang et al., 2024). There are numerous possible conformations that a protein can assume and, therefore, the folding process is susceptible to errors (Dobson, 2003; Bartlett; Radford, 2009). These misfolded, mistranslated, or excess proteins must be recognized and rapidly degraded to prevent the formation of toxic aggregates, since quality control systems are affected by the accumulation of defective proteins, which leads to the impairment of their functions or cell death (Tyedmers; Mogk; Bukau, 2010).

Proteins are synthesized in ribosomes as linear chains of amino acids and must fold into unique three-dimensional structures to fulfill their biological functions (Balchin; Hayer-Hartl; Hartl, 2016). During protein synthesis, the amount of production must be adjusted to the protein folding capacity of the cell, avoiding erroneous accumulation. In important life-prolonging pathways, such as caloric restriction, the improvement in proteostasis capacity is partly attributed to an overall reduction in the rate of protein translation (Taylor; Dillin, 2011).

In the dense cellular environment, newly synthesized proteins can misfold, forming toxic aggregates. To prevent this, different classes of molecular chaperones assist the emerging protein chains of the ribosome, guiding them to an efficient folding pathway (Balchin et al., 2016). Chaperones play a crucial role in maintaining protein homeostasis, facilitating proper protein folding and preventing protein aggregation (Montresor; Smith; Jones, 2024).

The cells developed a complex system capable of detecting and degrading abnormal proteins, avoiding proteotoxicity and ensuring the health of the cellular proteome. Thus, protein degradation is mediated by two main pathways: the ubiquitin-proteasome system (UPS) and autophagy (Varshavsky, 2017). These pathways play a critical role in removing misfolded or aggregated proteins. The ubiquitin-proteasome system is a selective proteolytic mechanism that marks target proteins with ubiquitin molecules, facilitating their degradation and, consequently, protein quality control (Glickman; Ciechanover, 2002). In turn, autophagy is an important pathway that enables the degradation, either on

a large scale or selectively, of proteins and the removal of protein aggregates within the cell (Yamamoto; Yue, 2014).

When the production of misfolded proteins exceeds the capacity of cellular quality control systems, a buildup of these proteins occurs, which can be toxic to cells. Related to this, many disease-associated proteins form amyloid fibrils, which are characterized by highly ordered cross-β leaf structures (Chiti; Dobson, 2017). These aggregated fibrils are a pathological feature of several neurodegenerative diseases, including Alzheimer's disease, Amyotrophic Lateral Sclerosis (ALS), and Parkinson's disease (Lackie et al., 2017).

In this context, the present study aimed to clarify how dysfunctions in autophagy pathways and in the ubiquitin-proteasome system are related to the development of neurodegenerative diseases.

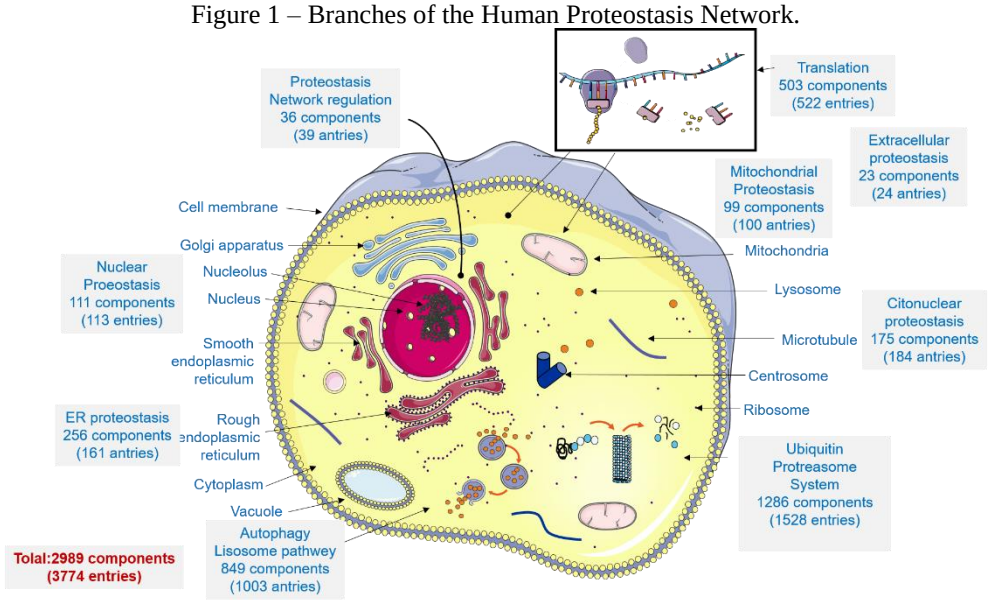
THEORETICAL FRAMEWORK

Proteostasis Network

Proteins are the backbone of cellular machinery, being responsible for maintaining the structure within the cell and enabling enzyme transport and signaling functions. Proteins must be correctly folded, in their native and functional state, expressed at adequate levels to be able to perform their functions, where they are often subjected to transcriptional and post-translational changes that allow the physiology of the protein to be altered, according to its needs. In addition, proteins must be located where their actions are needed (Llewellyn; Hubbard; Swift, 2024).

There are nine major divisions of the proteostasis network (Figure 1): mitochondrial proteostasis, endoplasmic reticulum (ER) proteostasis, cytonuclear proteostasis, nuclear proteostasis, cytosolic translation, proteostasis regulation, extracellular proteostasis, autophagy lysosomal pathway, and ubiquitin-proteasome system (UPS). The term cytonuclear refers to components that support proteostasis in both the cytosol and nucleus, while nuclear refers to components that primarily support nuclear proteostasis specifically. The list of non-redundant genes currently comprises about 3,000 members, while the total number of annotations is close to 3,800 (Hipp; Hartl, 2024).

Figure 1 – Branches of the Human Proteostasis Network.



Source: Adapted from Hipp; Hartl, 2024.

The proteostasis network can be divided into three distinct domains that serve proteins at different stages during their life cycle: biogenesis, conformational maintenance, and degradation. The first phase of biogenesis contains all the factors necessary for the transcription, translation, initial folding, and transport of a protein to the designated cell site. Conformational maintenance comprises the factors necessary to keep proteins in a functional state after their initial folding is complete. Finally, the degradation phase, which contains most of the factors, encompasses the machinery for the controlled degradation of functional and defective proteins (Hipp; Hartl, 2024). In order for proteins to perform their specialized function, they must achieve a favored native conformation. To fold correctly, proteins have the help of molecular chaperones and cofactors that will help establish folding stability. Protein biogenesis is quite complex and a protein can easily fold incorrectly, through several possible conformations, which are easily susceptible to errors (Hipp; Hartl, 2024).

Other factors also influence the conditions of protein synthesis, hindering protein folding, such as mutations, intrinsic and environmental effects, heat shock, metabolic stress and oxidative changes. Disruption of cellular homeostasis has implications for degenerative conditions, with growing evidence linking the accumulation of toxic proteins and aggregates to disease. These perturbations can be detrimental to cytosol proteins, an environment in which molecular crowding and the absence of lipid membranes mean that erroneous display of hydrophobic stains is energetically unfavorable (Baker; Bernardini, 2021).

In the cycle of protein biogenesis and degradation, synthesis generates a nascent polypeptide that folds until it reaches its native state. If misfolding occurs, the protein can be refolded, disaggregated, or directed for degradation through the ubiquitin-proteasome (UPS) system or autophagy. Protein

aggregates can form as off-path events and are also eliminated by autophagy to maintain cellular homeostasis (Sala; Bott; Morimoto, 2017).

Neurodegenerative diseases are marked by neuronal dysfunction and cell death, mainly caused by a specific group of proteins that form toxic aggregates. Among them are α-synuclein in Parkinson's disease, Tau protein in Alzheimer's disease and in several tauopathies, Huntingtin in Huntington's disease and SOD1 in some forms of Amyotrophic Lateral Sclerosis (ALS). In addition, other forms of aggregation are toxic, such as heterologous and artificial proteins responsible for part of the observed cytotoxicity (Hipp; Hartl, 2024).

These toxic aggregates of these proteins are thought to be small aggregates made up of a few protein units (oligomers), which are soluble and able to move within cells, making them more dangerous than large insoluble aggregates that have a fiber-like structure, a disordered fibrillar topology, and hydrophobic amino acid remnants exposed on unpaired beta strands. These soluble aggregates not only interact with lipid membranes, but also have a strong tendency to participate in anomalous interactions with other proteins, leading to their eventual sequestration in insoluble aggregate deposits. Several endogenous proteins, especially those newly synthesized or with large disordered regions, as well as specialized chaperones and proteasomes, have been identified as associated with these aggregates. Growing evidence indicates that confinement of oligomers in large insoluble deposits may provide relative protection, likely by decreasing the solvent-exposed interactive surface of these aggregates (Kinger *et al.*, 2023).

Protein Quality Control

The human body, under conditions of increased oxidative stress and, consequently, proteotoxic, creates an efficient mechanism to ensure the stability of the proteome, called the cellular protein quality control system, which aims to supervise the fine balance between protein folding and degradation (Baker; Bernardini, 2021). The specialized pathway for quality assurance of newly synthesized polypeptides—ER-associated protein degradation—operates within the endoplasmic reticulum and associated secretory pathways.

Molecular chaperones and additional proteins also act on these quality control pathways (Hetz, 2021). Molecular chaperones are the first to respond to imbalances in proteostasis by sequestering and attempting to catalyze the refolding of damaged proteins. Many of these chaperones are called Heat Shock Proteins (HSPs) due to their crucial role in the heat shock response pathway (Mogk *et al.*, 2018).

The ubiquitin-proteasome (UPS) system regulates the unique turnover rate of each cell protein. This process involves several hundred ubiquitin ligase E3 enzymes, which ensure specificity for each

substrate. Under certain physiological conditions, ubiquitin ligase E3 enzymes, along with adaptor proteins, can aid in the detection and targeting of abnormal and/or aggregated intracellular proteins to the alternative proteolytic pathway of autophagy, which is less specific than UPS and focuses primarily on removing cellular debris (Upadhyay, 2021).

Ubiquitin-Proteasome System (UPS)

The presence of misfolded proteins poses a serious risk to cells and means the waste of numerous resources. Although the toxic aggregates remain in the cell, their amino acids are not available to be recycled and used in the synthesis of new proteins. The ubiquitin-proteasome pathway is a multi-step process for protein degradation, in which multiple enzymes act in sequence to promote substrate proteolysis within a large cylindrical protein complex known as a proteasome. The 26S proteasome is a multi-subunit complex (it has multiple chains that associate to form the functional protein) containing a 20S central particle and one or two 19S regulatory subparticles to regulate the entry of ubiquitinated chains into the nucleus (Upadhyay, 2021).

The 20S central subunit contains three types of protease activities that govern the cleavage of the received polypeptides into smaller fragments. In the first phase, ATP-dependent, the enzyme E1 activates ubiquitin and transfers it to E2 through a transacylation reaction to a thiol group, forming a thioester bond. These thioesters (ubiquitin-E2 conjugates) provide ubiquitin molecules to a third class of enzymes called ubiquitin E3 ligases to tag substrate proteins. E3 then attaches ubiquitin to the target proteins for degradation. A single ubiquitin can be deposited on substrates (termed monoubiquitination), but often E3 ligases assemble chains with additional ubiquitins conjugated in a gradual manner to the N-terminal lysine or methionine residues of adjacent ubiquitin (multi-monoubiquitination and polyubiquitination). The binding patterns of subsequent ubiquitin moieties may govern the differential fates of target proteins. The complexity of this cascade is evident in the many unique E1, E2, and E3 enzymes that perform this process with the human genome encoding more than 600 distinct E3 ligases (Baker; Bernardini, 2021).

Autophagy

It is a lysosomal degradation mechanism that targets damaged organelles and different forms of cellular proteins, ubiquitinated or not. Autophagy has several pathways with different functions and specificities: macroautophagy (often referred to simply as autophagy), microphagy, and chaperone-mediated autophagy (CMA) (Filippone *et al.*, 2022). In macroautophagy, a double membrane-bound

structure (autophagosome) is formed and surrounds a large amount of cellular debris along with bulky protein inclusions (Upadhyay, 2021). Agrefagia is used to describe the selective targeting of bulky protein aggregates or inclusion bodies for degradation through macroautophagy in a process facilitated by adaptor proteins, such as P62, NBR1, and light chain 3 (LC3), a phagophore membrane receptor (Filippone *et al.*, 2022).

The process of macroautophagy can be divided into 4 steps: (1) induction, (2) formation of the autophagosome, (3) fusion with the lysosome or vacuole to form the autophagolysosome, (4) breaking of the encapsulated autophagic body, and recycling. The autophagosome forms when the phagophore closes and then fuses with late endosomal vesicles or lysosomal sacs. The internal contents of the new structure, called the amphisome, are then degraded by various lysosomal enzymes (Upadhyay, 2021).

Like agrefagia, some pathways of selective cytoplasmic protein degradation are mediated by HSC70 chaperones and their co-chaperones (Upadhyay, 2021). Microautophagy, for example, transports cytosolic proteins to vesicles using HSC70-dependent ESCRT I and III endosomal sorting complexes and involving invagination and tubular formation prior to gallbladder degradation. Chaperone-mediated autophagy (CMA) identifies proteins with the KFERQ motif by HSC70 and co-chaperones (Upadhyay, 2021). HSC70-conjugated substrates are internalized after binding to LAMP-2a (a lysosome-associated membrane protein) and subsequently degraded by membrane-bound proteases.

Chaperone-assisted selective autophagy (CASA) is also governed by the chaperones HSC70 and HSPB8, in conjunction with CHIP (an E3 ligase), which mediates the ubiquitination of proteins before their elimination in the lysosomal compartment in a P62-dependent manner (Bourdenx *et al.*, 2021).

Neuronal Autophagy

Neurons, together with glial cells, form the basis of the central nervous system (CNS), acting as specialized functional units in the reception, processing, and transmission of excitatory and inhibitory signals. They have a polarized structure, composed of dendrites and axons, and are post-mitotic cells, that is, they do not divide, they only differentiate. To ensure the proper functioning of the central nervous system, neurons must maintain their morphology and physiological functions, which depends on the recycling of damaged organelles (such as mitochondria and endoplasmic reticulum) and aggregated proteins, ensuring cellular homeostasis, neuronal health, and survival (Filippone *et al.*, 2022).

Neuronal autophagy is the main mechanism for recycling dysfunctional organelles and degrading toxic agents, especially macroautophagy, which, in addition to removing pathogens from within the cell, also acts on synaptic transmission. Neuronal macroautophagy involves the formation of autophagosomes, which fuse with lysosomes to degrade their content, with emphasis on autophagy-

related proteins such as ULK1/ULK2 and LC3, which are essential in this mechanism (Filippone et al, 2022). In neurons, the mammalian target of the rapamycin 1 complex (mTORC1) detects amino acids and intracellular nutrient levels and downregulates autophagy under conditions of nutrient abundance due to direct binding to the ULK1 and ULK2 protein complexes, due to the inhibitory phosphorylation of the two complexes preventing the activation of autophagy. In deficiency situations and under various cellular stresses, adenosine monophosphate-activated protein kinase (AMPK) activates autophagy and inhibits mTORC to control neuronal health (Bajaj *et al.,* 2018).

Mitophagy selectively degrades damaged mitochondria. It is regulated by the PINK1 protein, which recruits Parkin to initiate the breakdown of compromised mitochondria. This process is vital to prevent the accumulation of reactive oxygen species (ROS), protecting neurons from oxidative stress and neurodegeneration. Dysfunctions in these mechanisms contribute to diseases such as Parkinson's and Alzheimer's, associated with the accumulation of toxic proteins such as α-synuclein, Aβ, and tau (Filippone *et al.,* 2022).

METHODOLOGY

For the elaboration of the work, a survey of scientific data was carried out, based on the analysis of articles and literature related to the theme. Scientific articles related to proteostasis alterations and neurodegenerative diseases were obtained by direct search in indexed databases, using the search terms: "proteostasis", "ubiquitin-proteasome system", "autophagy" and "neurodegenerative diseases". Scientific articles on the subject were accessed in the MEDLINE (via Pubmed), Science direct (via Elsevier), CAPES periodicals and LILACS databases.

A descriptive approach was carried out on the alterations in the function of components of the ubiquitin-proteasome system and autophagy pathways, which may be related to the generation/progression of neurodegenerative diseases. Studies that evaluated the physiological functions of the ubiquitin-proteasome system and autophagy pathways, as well as the disruption of these factors and the consequent effects on the prevalence of neurodegenerative diseases in humans were included and those that showed the relationship between the dysfunction of these protein quality control pathways and any other disease were excluded from the study.

To select and manage all the references found in the databases, the Rayyan software (Qatar Computing Research Institute, Doha, Qatar) was used, which organized the references and identified all duplicates, which were manually removed. Some studies that were untitled, which were identified as duplicates, were added for later reading and selection. Studies were independently evaluated by titles and abstracts to initially select those that met the eligibility criteria. After excluding studies that did not

meet the criteria, the selected studies were read thoroughly to confirm that they were related to the eligibility criteria.

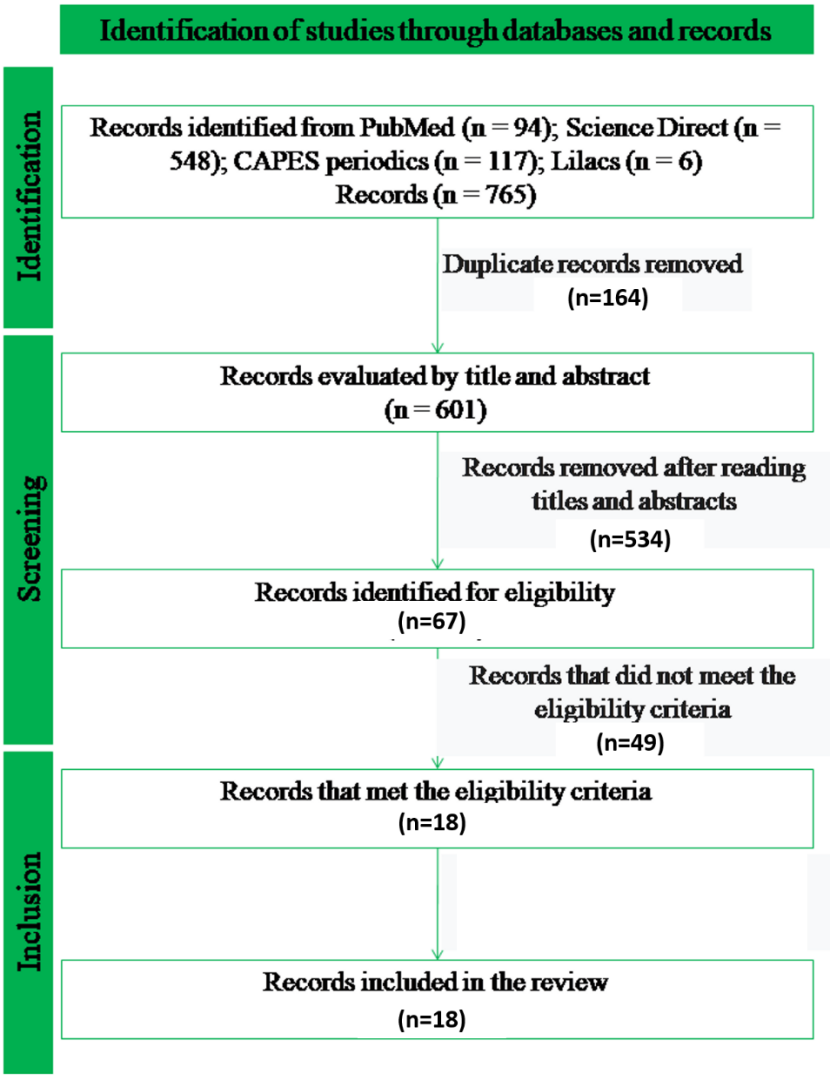
The information was reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020, as this statement provides guidance for updated reporting for systematic reviews, which reflect advances in methods for identifying, selecting, evaluating, and synthesizing studies (Page; Mckenzie; Bossuyt, 2022).

RESULTS AND DISCUSSION

Analysis of References Found in The Databases

Several studies have investigated the disruption of proteostasis in the generation of neurodegenerative diseases. This scope review aimed to map studies that explain and relate this protein homeostasis disorder and a defect in the protection of heat shock proteins in the generation of neurodegenerative diseases. Of the 765 studies initially found, after all the steps described in the flowchart in Figure 2, 18 references were read in detail to prepare the present study (Figure 2).

Figure 2: PRISMA flowchart of the study selection process



Source: Flowchart constructed by the authors.

Neurodegenerative Diseases and Ubiquitin-Proteasome System (UPS)

After a failed attempt to refold proteins, the chaperones send these proteins for degradation with the aid of ubiquitin ligases E3. The most evident of the ligases is the C-terminal of the interactive protein Hsc70 (CHIP) or STUB1 which uses its U-box domain, along with chaperones, to ubiquitinate damaged or misfolded proteins. The phosphorylation of CHIP by protein kinase G increases CHIP activity with chaperones (Baker; Bernardini, 2021).

CHIP defects have been identified in several neurodegenerative diseases and neuronal decline, such as Alzheimer's disease, because it and its bound chaperone (Hsc70) direct phosphorylated tau (related to the formation of neurofibrillary tangles in Alzheimer's) for degradation by the ubiquitin-proteasome system (UPS), preventing its pathological aggregation. Mutations in CHIP, especially in its

catalytic U-box domain or in the domain that interacts with chaperones, highlight its importance in proteostasis. Another ligase, UBR4, would be related to neurodegeneration, as in Parkinson's disease, involved in the processing of PINK1 and helping in the regulation of activity and/or stability. Alterations in UBR4 can negatively affect the function of PINK1, contributing to neurodegeneration (Baker; Bernardini, 2021).

The substrates sent for degradation touch the proteasome and bind to the ubiquilin family and direct proteins for digestion. Defects in the processing of substrates by ubiquilin-2 are related to problems in ALS. Probably, the toxic aggregates in ALS reach the proteasome, but are resistant to unfolding; in this way, they obstruct the degradation system and make it difficult for other substrates to enter (Kinger *et al.,* 2023). Ubiquitin deubiquitinses (DUB) or ubiquitin proteases remove ubiquitins from substrates and target misfolded proteins after heat shock (Baker; Bernardini, 2021). The deubiquitinase ataxin-3, specifically, when bound to CHIP, is related to Machado-Joseph disease (or spinocerebellar ataxia type 3), where abnormal expansion of glutamine (poly-Q) repeats in the protein occurs. In vivo experiments have shown that the inhibitory effect of poly-Q does not occur directly in the proteasome, but indirectly, from the sequestration and functional depletion of essential components of the proteostasis network (Kinger *et al.,* 2023).

It was discovered that the expansion of an exon fragment of the huntingtin gene by poly-Q stabilizes misfolded proteins, which would be degraded through UPS or would be aggregated as metastable proteins. Related to this, mutant huntingtin has been shown to interrupt folding, as well as influence the specific tasks of chaperones and the conformational maintenance of endogenous or exogenously expressed metastable proteins. In addition, it can also cause the ubiquitination of the molecular chaperones themselves (Wagner *et al.*, 2024).

Mutants of the TDP-43 protein, associated with ALS, along with chaperones, would be accumulated in specific compartments such as nucleolus or anisomes. Both defective TDP-43 and Superoxide Dismutase 1 (SOD1) can be sequestered in the mitochondria to prevent aggregation. Anisosomes have chaperones in their core and convert to insoluble aggregates when chaperones deplete ATP (Kinger *et al.,* 2023).

Molecular chaperones act in phase separation and function to maintain a liquid, non-fibrillar state, and in addition to TDP43, phase separation has also been observed for other proteins connected to protein misfolding diseases, including Tau (Alzheimer's disease), α-synuclein (Parkinson's disease), and Htt (Huntington's disease). The robust, highly interconnected, and redundant structure of the proteostasis network enables affected cells to endure the harmful effects of anomalous proteins—in some cases, decades in humans, for extended periods of time. This contributes to the late onset of the disease, even

with the continuous production of mutant proteins throughout life, as seen in poly-Q expansion diseases (Hipp; Hartl, 2024).

Neuronal Autophagy Dysfunctions in Alzheimer's and Parkinson's Diseases

Autophagy dysfunction is related to the aggregation of abnormal proteins such as α-synuclein (the main component of Lewy's body), Aβ, and tau (main components of amyloid plaques and neurofibrillary tangles). The accumulation of α-synuclein protein (α-syn) in Lewy bodies (CL) is a hallmark of Parkinson's disease (PD). This accumulation impairs the lysosomal autophagy pathway, causing failures in the degradation of damaged organelles and proteins (Filippone *et al.*, 2022).

Spencer et al. (2009) showed that neuronal cells infected with lentiviruses (used to introduce genes that express α-synuclein) accumulated autophagic vesicles positive for lysosomal/autophagy markers such as cathepsin D and LC3, and had an increase in the expression of the Beclin-1 protein, suggesting that α-syn accumulation alters the autophagic degradation pathway in these cells.

Thus, it was observed that the overexpression of α-syn increases the levels of autophagic vesicles and reduces lysosomal efficiency, which can be partially reversed by rapamycin, which activates autophagy by inhibiting mTOR. Specific gene mutations, such as A30P and A53T in α-syn, accelerate the formation of protofibrils that compromise lysosomes. Overexpression of α-syn in neuroblastoma cells generates alterations in Ca2+ signaling, decreased expression of the LAMP1 protein, alkalinization of lysosomal pH, and increased vesicles. Other mutations, such as in leucine-rich kinase 2 (LRK2), affect autophagosome transport into neurons and loss of motility, while mutations in PINK1 and Parkin impair mitophagy, resulting in the accumulation of dysfunctional mitochondria and oxidative stress, also associated with mitochondrial Ca2+ concentration imbalance. These dysfunctions contribute to the degeneration of dopaminergic neurons, aggravating the progression of PD (Filippone *et al.*, 2022).

Mutations in the SORL1 receptor encoding gene, which regulates protein trafficking between the trans-Golgi network and endosomes, increase the risk of late-onset Alzheimer's. The loss of SORL1 increases the production of the Aβ peptide, by favoring the β-secretase-mediated pathway, and prevents the formation of autophagosomes, evidenced by the regulation of the LC3II marker (Zatyka; Sarkar; Barret, 2020). In addition, the reduction of autophagy factor beclin-1 contributes to the accumulation of Aβ plaques, but its induction can decrease these plaques. Aβ oligomers formed in the early stages of the disease generate a toxic environment, leading to the formation of dystrophic neurites, which impair neuronal function. The ATG9A protein, an early autophagy protein involved in the early formation of preautophagosomes, is found accumulated around Aβ plates, reinforcing its relationship with the development of these structures (Filippone *et al.*, 2022).

The ATG5 and ATG7 genes are related to spontaneous degeneration associated with motor impairment, neuronal loss, and early death. Studies of cell culture and behavioral work in mice have shown regulation of adult neuronal stem cells by autophagy, impacting axonal growth, synaptic assembly, and dendritogenesis. Reduced autophagy in the hippocampus and astrocytes resulted in cognitive problems; meanwhile, ATG7 is related to the regulation of synaptosome degradation, impacting synaptic protein expression, dendritic spine density, and neuronal connectivity (Hetz, 2021).

Measures aimed at increasing CMA, such as the activation or overexpression of LAMP2, have the potential to offer protection against several neurodegenerative diseases associated with aging. Evidence of this has been demonstrated in PD models and in other tissues such as the liver (Hetz, 2021). In a study conducted on mice, CMA deficiency in excitatory neurons is related to negative effects on motor behavior, memory, and life expectancy, highlighting the importance of CMA in maintaining neuronal health and preventing neurodegenerative diseases (Bourdenx *et al.*, 2021).

CMA targets only soluble proteins, which are recognized and misfolded by Hsc70 coupled to the lysosomal receptor LAMP2A for lysosome-mediated degradation (Hetz, 2021). The substrate/chaperone complex is attached to the tail of this protein, generating its multimerization for protein transport until degradation. LAMP2A is the only isoform, of the three protein variants of the Lamp2 gene, that participates in CMA. After removal of LAMP2A in the neurons of mice, it was observed that CMA plays a crucial role in the maintenance of the neuronal proteome in vivo under normal and pathological conditions (Bourdenx *et al.,* 2021).

Evidence indicates that chemical activation of CMA may be a promising therapeutic strategy for neurodegenerative disorders. Unlike other cell types, neurons do not exhibit compensatory activation of macroautophagy when CMA is blocked, highlighting the importance of this specific pathway in proteome preservation. In neurons, inhibition of CMA results in the trapping of glycolytic enzymes in aggregates, reducing their activity, while in other cells these enzymes remain functional. CMA is essential for degrading soluble proteins and preventing the formation of toxic aggregates. Their deficiency leads to the accumulation of supersaturated proteins, which are more prone to misfolding and aggregation. In experimental models, gradual blockade of CMA induced protein changes without immediate neurodegeneration, which suggests that these models may represent early stages of neurodegenerative diseases. In Alzheimer's, early inhibition of CMA is associated with accumulation of tau pathology, and activation of CMA has been shown to significantly reduce tau and β-amyloid pathologies, even in advanced stages of the disease. These results suggest that CMA can have a relevant therapeutic impact, including on preexisting pathologies, since, when administering the CMA activator in the models, there was a reduction in the number and size of mature amyloid depositions (Bourdenx *et al.*, 2021).

CONCLUSION

This study addressed the central mechanisms involved in the disruption of the ubiquitin-proteasome system and the mechanisms of autophagy in the development/progress of neurodegenerative diseases. The research revealed that failure in these protein quality control pathways is closely associated with the accumulation of misfolded proteins, such as α-synuclein, superoxide dismutase, tau, and Aβ, which form toxic aggregates in diseases such as Alzheimer's, Parkinson's, ALS, and Huntington's.

The findings pointed to the need for therapeutic strategies that precisely modulate stress response pathways. In any case, further additional studies are needed to optimize these interventions, looking for diagnostic biomarkers, propensity, and/or progression to these diseases, as well as developing more effective therapies for these debilitating conditions.

REFERENCES

BAJAJ, L. et al. Lysosome biogenesis in health and disease. **Journal of Neurochemistry**, vol. 148, no. 5, p. 573-589, 2018.

BAKER, H.A.; BERNARDINI, J. P. It is not just a phase; quality-controlled ubiquitination of cytosolic proteins. **Biochemical Society Transactions**, vol. 49, no. 1, p. 365–377, 2021.

BALCH, W. E. et al. Adapting proteostasis for disease intervention. **Science**, vol. 319, no. 5865, p. 916-919, 2008.

BALCHIN, D.; HAYER-HARTL, M.; HARTL, F. U. In vivo aspects of protein folding and quality control. **Science**, vol. 353, no. 6294, p. 871-876, 2016.

BARTLETT, A. I.; RADFORD, S. E. An expanding arsenal of experimental methods yields an explosion of insights into protein folding mechanisms. **Nature Structural & Molecular Biology**, v. 16, no. 6, p. 582–588, 2009.

BOURDENX, M. et al. Chaperone-mediated autophagy prevents collapse of the neuronal metastable proteome. **Cell**, vol. 184, n. 10, p. 2696-2714, 2021.

CHITI, F.; DOBSON, C. M. Protein misfolding, amyloid formation, and human disease: a summary of progress over the last decade. **Annual Review of Biochemistry**, vol. 86, p. 27-68, 2017.

DOBSON, C. M. Protein folding and misfolding. **Nature**, vol. 426, no. 6968, p. 884–890, 2003.

FILIPPONE, A. et al. The contribution of altered neuronal autophagy to neurodegeneration. Pharmacology & Therapeutics, vol. 238, p. 108178, 2022.

GLICKMAN, M. H.; CIECHANOVER, A. The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. **Physiological Reviews**, vol. 82, no. 2, p. 373-428, 2002.

HETZ, C. Adapting the proteostasis capacity to sustain brain healthspan. **Cell**, vol. 184, n. 6, p. 1545-1560, 2021.

HIPP, M. S.; HARTL, F. U. Interplay of proteostasis capacity and protein aggregation: implications for cellular function and disease. **Journal of Molecular Biology**, vol. 436, no. 14, p. 168615, 2024.

KINGER, S. et al. Molecular chaperones' potential against defective proteostasis of amyotrophic lateral sclerosis. **Cells**, vol. 12, no. 9, p. 1302, 2023.

LACKIE, R. E. et al. The HSP70/HSP90 chaperone machinery in neurodegenerative diseases. **Frontiers in Neuroscience**, vol. 11, p. 1-23, 2017.

LLEWELLYN, J.; HUBBARD, S. J.; SWIFT, J. Translation as an emerging constraint to protein homeostasis in aging. **Trends in Cell Biology**, vol. 34, no. 8, p. 646-656, 2024.

MOGK, A.; BUKAU, B.; KAMPINGA, H. H. Cellular handling of protein aggregates by disaggregation machines. **Molecular Cell**, vol. 69, no. 2, p. 214-226, 2018.

MONTRESOR, P.; SMITH, J. A.; JONES, R. L. HSP110 is a modulator of amyloid beta (Aβ) aggregation and proteotoxicity. **Journal of Neurochemistry**, vol. 00, p. 1-19, 2024.

PAGE, M. J.; MCKENZIE, J. E.; BOSSUYT, P. M. et al. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 explanation and elaboration: update guidance and exemplars for reporting systematic reviews. **British Medical Journal**, vol. 372, no. 160, p. 1-36, 2021.

SALA, A.J.; BOTT, L. C.; MORIMOTO, L.C. Shaping proteostasis at the cellular, tissue, and organismal level. **Journal of Cell Biology**, vol. 216, no. 5, p. 1231-1241, 2017.

SPENCER, B. et al. Beclin 1 gene transfer activates autophagy and ameliorates the neurodegenerative pathology in alpha-synuclein models Parkinson's and Lewy body diseases. **Journal of Neurosciences**, vol. 29, no. 43, p. 13578-13588, 2009.

TAYLOR, J. P.; DILLIN, A. Aging as an event of proteostasis collapse. **Cold Spring Harbor Perspectives in Biology**, vol. 3, no. 5, p. a004440, 2011.

TYEDMERS, J.; MOGK, A.; BUKAU, B. Cellular strategies for controlling protein aggregation. **Nature Reviews Molecular Cell Biology**, vol. 11, no. 11, p. 777-788, 2010.

UPADHYAY, A. Natural compounds in the regulation of proteostatic pathways: An invincible artillery against stress, aging, and diseases. **Acta Pharmaceutica Sinica B**, v. 11, no. 10, p. 2995-3014, 2021.

VARSHAVSKY, A. The ubiquitin system, autophagy, and regulated protein degradation. **Annual Review of Biochemistry**, vol. 86, p. 123-128, 2017.

WAGNER et al. General loss of proteostasis links Huntington's disease to Cockayne syndrome. **Neurobiology of Disease**, vol. 201, p. 106668, 2024.

YAMAMOTO, K.; YUE, Z. Autophagy and its normal and pathogenic states in the brain. Annual **Review of Neuroscience**, vol. 37, p. 55-78, 2014.

ZATYKA, M.; SARKAR, S.; BARRETT, T. Autophagy in rare (non-lysosomal) neurodegenerative diseases. **Journal of Molecular Biology**, vol. 432, no. 8, p. 2735-2753, 2020.

ZHANG, J.; et al. CaMKII suppresses proteotoxicity by phosphorylating BAG3 in response to proteasomal dysfunction. **EMBO Reports**, vol. 25, no. 14, p. 4488-4514, 2024.