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Review

Heat Shock Proteins in Neurodegenerative Diseases

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ABSTRACT: Neurodegenerative diseases (NDs), which affect millions globally, are characterized by progressive motor and non-motor deficits and currently lack a cure. Molecular chaperones, particularly heat shock proteins (HSPs), have emerged as promising therapeutic candidates to combat these conditions. HSPs are classified into six major families and have been extensively studied in contexts ranging from autoimmune diseases to cancer and viral infections. Their broad functional repertoire—which includes preventing protein aggregation, correcting misfolding, regulating apoptosis, mediating autophagy, and maintaining proteostasis—positions them as potent modulators of the pathological processes underlying NDs. This review will explore the mechanisms of different HSP classes and critically assess their therapeutic potential for NDs.

Keywords: Neurodegenerative disease, heat shock proteins, protein aggregation, therapeutics

1. Introduction

Neurodegenerative diseases (NDs) are characterized by the progressive degeneration of specific neuronal populations and the accumulation of pathological protein aggregates. Major categories include amyloidoses, tauopathies, synucleinopathies, and TAR DNA-binding protein 43 (TDP-43) proteinopathies, which frequently exhibit overlapping neuropathological features. Currently, the definitive diagnosis often relies on postmortem examination [1]. The structural and functional impairment of neurons disrupts communication within neural circuits, leading to motor, sensory, and cognitive deficits [2]. Although these disorders range from common conditions such as Alzheimer's disease (AD) and Parkinson's disease (PD) to rare entities like neuronal intranuclear inclusion disease (NIID), effective treatments remain limited [3].

A central pathological feature of NDs is the accumulation of toxic protein aggregates, resulting from impaired clearance mechanisms, which contributes to neuronal damage and death. Consequently, therapeutic strategies aimed at reducing or clearing these aggregates hold significant promise [4].

Heat shock proteins (HSPs) represent a family of molecular chaperones that play essential roles in maintaining protein homeostasis. Under both normal and stress conditions, HSPs assist in refolding misfolded proteins or facilitate their degradation, thereby counteracting aggregation [5]. Given their protective functions, enhancing HSP activity presents a promising therapeutic avenue for NDs. This review will systematically examine the classification and roles of

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HSPs in neurodegeneration and discuss emerging HSP-targeted treatment strategies.

2. Structure, Functions, and Mechanisms

2.1. Heat Shock Response and Heat Shock Proteins

Cells are sensitive to their growth conditions; they often encounter harsh environments—such as toxins, chemicals, and stress—that impair their function and can lead to cell death[6]. In response, cells activate the heat shock response (HSR), a conserved protective mechanism that temporarily halts protein synthesis to prevent further damage and support survival [7]. HSR can also be triggered during the normal cell cycle under non-stress conditions to aid cellular development and growth [8].

Heat shock factor 1 (HSF1) is a key regulator of the HSR. Under normal conditions, HSF1 exists as an inactive monomer in the cytoplasm, bound to inhibitory chaperones, including HSP90, HSP70, and the TRiC complex. Upon stress, HSF1 dissociates from these inhibitors, trimerizes, and translocates to the nucleus, where it binds to heat shock elements (HSEs) and activates transcription of HSPs. This activation is tightly regulated by post-translational modifications (PTMs). Acetyltransferase EP300 enhances HSF1 stability by acetylating lysines 208 and 298, while acetylation of lysine 80 inhibits DNA binding by disrupting interaction with the phosphate backbone.

Additionally, HSF1 activity is modulated by phosphorylation, sumoylation, and ubiquitination, which collectively regulate its transcriptional activity and turnover [9, 10]. Mitochondria generate ROS during energy production, and their accumulation in the nucleus under hypoxia creates oxidative stress, activating hypoxia-induced factor (HIF)-1α. Heat stress also increases HIF-1α, which induces the HSR and upregulates HSF1. Under hypoxia, HIF-1α promotes HSF gene transcription by binding hypoxia response elements, and its knockdown reduces HSF levels [11].

HSPs are classified by molecular weight into major families, including HSP100, HSP90, HSP70, HSP60, HSP40, and small HSPs, each comprising multiple members [9, 12]. Their primary function is to maintain proteostasis through chaperone activities such as protein refolding, disaggregation, and degradation, while also regulating apoptosis and signal transduction [9, 12]. In the central nervous system (CNS), key expressed HSPs—such as HSP105, HSP90, HSP70, DNAJA2 (HSP40), and small HSPs (e.g., HSPB1, HSPB5, HSPB8)—are vital for neuronal viability and synaptic function (Fig. 1) [13, 14].

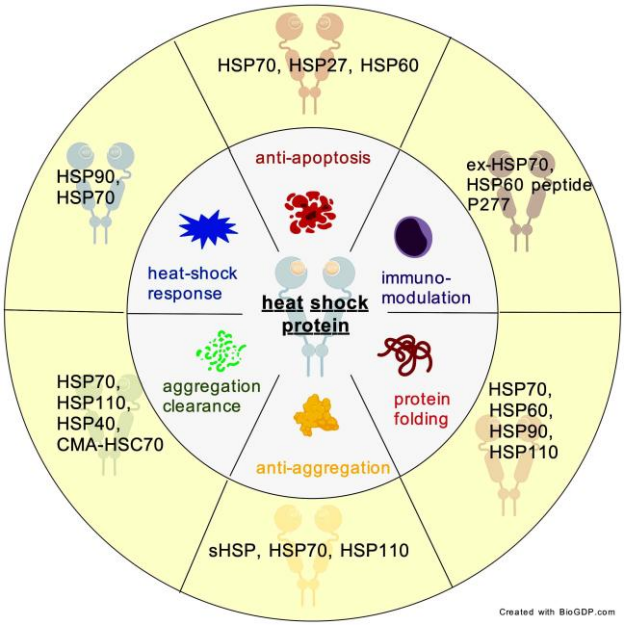

Figure 1. The Biological Functions of Heat Shock Proteins. This figure depicts six coordinated defense layers against protein toxicity in neurodegenerative diseases: Folding/refolding: HSP70 isoforms (HSPA1A/HSC70) drive ATP-regulated cycles via N-terminal ATPase and C-terminal substrate-binding domains. HSP60 forms mitochondrial double-ring barrels with HSP10 for encapsulated folding; HSP90 stabilizes client kinases/receptors; HSP110 serves as a nucleotide-exchange factor (NEF) that reactivates HSP70. Anti-aggregation: Small HSPs (sHSPs; HSPB1–HSPB10) function as ATP-independent holdases, binding hydrophobic patches via their α-crystallin domain to prevent oligomer progression. HSPB5 (αB-crystallin) and HSPB1 block α-synuclein/Aβ fibrillization in PD and AD. Disaggregation/clearance: HSP104 (AAA+ ATPase) extracts polypeptides from aggregates with HSP70/HSP40. HSC70 delivers KFERQ-motif proteins to lysosomal LAMP2A for chaperone-mediated autophagy (CMA). CHIP ubiquitinates irreparable clients for proteasomal degradation. HSP90 inhibitors (geldanamycin) promote this by releasing HSF1. HSF1 regulation: Under basal conditions, HSF1 monomers are sequestered by HSP90/HSP70. Proteotoxic stress triggers trimerization, nuclear translocation, and heat shock element (HSE) binding, upregulating HSP genes. Post-translational modifications (acetylation, phosphorylation) fine-tune activity. Co-inducers like Arimoclomol amplify HSF1 DNA binding. Anti-apoptotic signaling: HSP70 blocks caspase activation and mitochondrial permeabilization; HSP27 stabilizes membranes; TRAP1 prevents mPTP opening. Declining levels with age remove this cytoprotective buffer. Extracellular immunomodulation: HSPs (especially HSP70) are released via exosomes or lysosomal escape, acting as damage-associated molecular patterns (DAMPs). They can activate microglia via TLRs (pro-inflammatory) or sequester extracellular Aβ oligomers (neuroprotective). EV-mediated delivery enables intercellular chaperone transfer. Abbreviations: HSP – Heat shock protein; HSC70 – Heat shock cognate 70; HSF1 – Heat shock factor 1; HSR – Heat shock response; CMA – Chaperone-mediated autophagy

2.2. HSP100

HSP100 (also called Clp) is a family of ATP-dependent chaperone proteins that act as molecular machines to remodel other proteins. The HSP100/Clp family of AAA+ chaperones functions as a conserved mechanical platform for protein remodeling, utilizing ATP hydrolysis to translocate polypeptides through a central pore in their hexameric ring [15, 16]. This core threading mechanism is specialized through structural classification into Class 1 proteins, the disaggregases ClpB and HSP104, which possess two nucleotide-binding domains and a regulatory M-domain, and Class 2 proteins, such as the unfoldases ClpX and ClpA, which contain a single nucleotide-binding domain (NBD) and partner with the ClpP peptidase for targeted proteolysis [17, 18]. The system's functional diversity is dramatically expanded by adaptor proteins (e.g., SspB, ClpS, and MecA), which tether specific substrates to the chaperone, and anti-adaptors, which provide regulated inhibition, enabling precise control over cellular processes such as competence development and stress responses.

The specialized activity of the disaggregases is exquisitely regulated through partnership with the HSP70 system. The unique M-domain of ClpB/HSP104 acts as a molecular toggle switch; HSP70 binding releases its autoinhibitory contact with the AAA-1 ring, activating the ATPase to extract proteins from aggregates selectively. This gating mechanism ensures activity is confined to aggregate surfaces, preventing deleterious unfolding of native proteins [19]. For Class 2 chaperones, the functional partnership with ClpP creates a proteolytic complex where substrates are unfolded and translocated into ClpP's proteolytic chamber for degradation, a process essential for protein quality control and regulatory turnover [16].

Pathogens often require HSP100s for survival within a host, and their absence in humans provides a therapeutic window. This is exemplified by acyldepsipeptide (ADEP) antibiotics, which lethally activate ClpP independently of its HSP100 partners, causing uncontrolled proteolysis and validating this system as a promising drug target [20].

2.3. HSP90

HSP90 is localized in three distinct cellular compartments: HSP90A and HSP90AB in the cytoplasm, glucose-regulated protein (GRP) 94 in the endoplasmic reticulum (ER), and tumor necrosis factor receptor-associated protein 1 (TRAP1) in the mitochondria [12].

HSP90 comprises three conserved domains: an N-terminal domain (NTD) with an ATP-binding pocket, a middle domain (MD) that binds substrates and modulates ATPase activity, and a C-terminal domain (CTD) that mediates dimerization and interactions with co-factors [21, 22]. Its chaperone function is ATP-dependent and regulated by co-chaperones. In the ATP-free state, HSP90 adopts an open conformation to recruit client proteins (e.g., tau, α-synuclein (α-syn), huntingtin, kinases) via its MD [23, 24]. ATP binding to the NTD induces a conformational change, leading to NTD dimerization and lid closure, thereby activating ATP hydrolysis and facilitating client protein maturation, activation, or degradation [22].

HSP90 mainly assists in protein folding and prevents protein aggregation. Several signaling pathways, especially those involving protein kinases and steroid hormone receptors, are connected to cytosolic HSP90. It also supports cytoskeletal integrity by functioning as a ‘molecular glue’ due to its adhesive properties. GRP94 manages ER stress by boosting the unfolded protein response (UPR), increasing the ER’s folding capacity, reducing apoptosis, and easing metabolic stress. Additionally, TRAP1 preserves mitochondrial integrity and shields cells from mitochondrial apoptosis [21].

2.4. HSP70

HSP70 is a 70 kDa molecular chaperone found in various cellular compartments, including HSPA1A, HSPA2, HSPA6, and HSPA8 in the cytosol, HSPA5 in the ER, and HSPA9 in the mitochondria [13]. HSP70 consists of an N-terminal NBD, a conserved linker, and a C-terminal substrate-binding domain (SBD). The NBD shares structural similarity with actin and contains the ATP-binding site, while the non-specific SBD enables interaction with diverse peptide substrates [25]. Its chaperone activity is driven by an ATP-regulated cycle between open and closed conformations. ATP binding to the NBD induces an open state, where the SBD's α-helical "lid" is open, allowing for rapid substrate binding and release. Subsequent substrate binding stimulates ATP hydrolysis, triggering a transition to a closed conformation where the lid closes over the β-sheet, trapping the substrate with high affinity to facilitate its refolding [25, 26]. HSP70 and HSP90 jointly regulate key signaling regulators, ensuring their full activation in response to upstream signals and their repression in their absence [26]. Studies have also shown that the overexpression of HSP70 confers resistance to apoptosis-inducing agents, such as tumor necrosis factor, doxorubicin, and staurosporine [27].

Heat Shock Cognate 70

HSC70 (heat shock cognate 70 kDa protein) acts as the primary chaperone in chaperone-mediated autophagy (CMA). It specifically detects cytosolic proteins with a

KFERQ-like motif and guides them to the lysosomal membrane. At the membrane, the substrate-chaperone complex interacts with LAMP2A, a single-span lysosomal receptor. This process is tightly regulated by a network of co-chaperones that bind to HSC70. HSC70-interacting protein (HIP) enhances the stability of the HSC70-substrate complex. HSP40, a J-domain co-chaperone, increases HSC70's ATPase activity to improve substrate binding and processing. The adapter protein HOP (HSP70-HSP90 organizing protein) recruits HSP90 into the complex; HSP90 then helps prevent protein aggregation and stabilizes the LAMP2A translocation complex during multimerization. Conversely, Bcl-2-associated athanogene-1 acts as a nucleotide exchange factor, typically promoting substrate release from HSC70 and possibly directing clients to proteasomal degradation. This regulation modulates HSC70's role in CMA [28-31].

CMA requires two key components: lysosomal lumen HSC70 (ly-HSC70) and the receptor LAMP-2A. LAMP-2A, the sole CMA receptor, is essential, as its knockout abolishes CMA activity. Its levels decline with age, contributing to reduced CMA and protein aggregation [28]. The CMA process begins when cytosolic HSC70 recognizes a substrate and delivers it to LAMP-2A on the lysosomal membrane. The substrate is then unfolded and translocated into the lysosome through a LAMP-2A translocon with the assistance of ly-HSC70. Inside, the substrate is rapidly degraded, while cytosolic HSC70 is recycled for a new cycle [28].

HSP110 and GRP170

HSP110 and GRP170 have high molecular weights due to a loop structure and belong to the HSP70 superfamily. These HSP110 and GRP170 interact with the immune system and exhibit strong protein-binding properties. They work alongside other HSPs to promote cell survival by restoring protein folding and cellular functions (Table 1) [32].

Table 1. HSP 110, HSP170, and their features.

	HSP110	GRP170 / HSP170
Alternative names	HSP105α/β, Apg-1, Apg-2, HSPA4	Orp150, HYOU1, HSP170
Molecular weight	≈ 110 kDa	≈ 170 kDa
Super-family	HSP70 super-family	
Main localization	Cytosol (constitutive & stress-inducible); nucleus (β-isoform)	Endoplasmic reticulum (ER); mitochondria; extracellular upon stress
Typical inducers	Heat shock, oxidative stress, ethanol, anoxia-reperfusion, inflammation	Glucose starvation, hypoxia, ER-calcium depletion, viral infection
Chaperone activity	Holdase & refolding of heat-denatured proteins; disaggregase with HSP70/HSP40	Holdase & refolding in ER; quality control of secretory proteins
Co-chaperone role	Nucleotide-exchange factor (NEF) for HSP70/HSC70	NEF for ER-HSP70 (GRP78/BiP)
Immune functions	DAMP Enhances antigen cross-presentation Stimulates DCs & cytokine release (IL-6, IL-12, CD40)	DAMP Chaperones antigens for MHC-I cross-presentation Potentiates TLR9 signalling (binds CpG-ODN)
Cancer relevance	Frequently over-expressed; stabilises oncogenic proteins (e.g., β-catenin); prognostic marker for poor survival	Up-regulated in tumours; supports tumour-cell survival; vaccine vehicle
Neurodegeneration	Prevents aggregation of mutant SOD1 & hyper-phosphorylated tau; protects against TBI	Protects neurons from hypoxia/ischemia; mediates neuro-protective sAPPα response
Inflammatory & metabolic diseases	Modulates CD1d-NKT axis in gut; antibody target in MS; delays ulcer healing	Improves insulin sensitivity; auto-antibody target in T1D; protects lung & heart from ischemic injury yet may promote fibrosis

Abbreviations: HSP: heat shock protein, GRP: glucose-regulated protein, DC: dendritic cells, MHC: major histocompatibility complex, SOD1: superoxide dismutase 1, TBI: traumatic brain injury, DAMP: damage-associated molecular pattern, NKT: natural killer T cells, MS: multiple sclerosis, T1D: type 1 diabetes, sAPPα: soluble amyloid precursor protein alpha, TLR9: Toll-like receptor 9.

HSP110 plays a dual role in proteostasis, functioning both as a holdase that directly prevents protein aggregation and as a nucleotide exchange factor (NEF) that supports HSP70-mediated folding and refolding [33]. Structurally, HSP110 shares significant homology with HSP70, comprising a NBD, a β-sheet domain, and a three-helix bundle, which underpin its chaperone functions [34]. It exists in two major isoforms: the cytosolic HSP105α, induced by diverse stressors, and the nuclear HSP105β, whose expression is triggered explicitly by heat [32].

Despite their evolutionary relationship, HSP110 and HSP100 exhibit distinct mechanistic roles. HSP110 primarily serves as a potent NEF for HSP70, accelerating ADP/ATP exchange to regulate the HSP70 chaperone cycle and facilitate the release of client proteins [34]. In contrast, the HSP100 family member HSP104 functions as an AAA+ ATPase disaggregase. It assembles into a hexameric translocase that uses mechanical ATP hydrolysis to thread and extract polypeptides from aggregates [35]. Thus, while HSP110 acts indirectly by enhancing HSP70 activity, HSP100 operates as a direct disaggregation motor. Notably, these systems can function collaboratively: in yeast, the HSP70 system (augmented by its NEF) recognizes aggregated substrates and presents them to HSP104, which then performs ATP-dependent translocation and disaggregation [36].

Another key chaperone, GRP170, belongs to the HSP70 family and plays roles in protein folding and protection against heat-induced aggregation [32, 37]. GRP170 is localized to the ER by a C-terminal retention signal [32], and its expression is induced by diverse ER stressors such as glucose starvation, hypoxia, and viral infection. While its overall domain organization (NBD, β-sheet SBD, and C-terminal α-helical region) is conserved with HSP70 and HSP110, GRP170 possesses a unique C-terminal structure [37].

2.5. HSP60

HSP60, also known as chaperonin 60 [7, 38] consists of three domains: the apical domain, which binds to substrate proteins and co-chaperones; the intermediate domain, which serves as a flexible linker between the apical and equatorial domains; and the equatorial domain, located at the base of the ring structure, which promotes interactions among single subunits within a ring and between two heptameric rings [38, 39]. HSP60 is predominantly localized in the mitochondria (80-85%), and disruption of HSP60 function can lead to mitochondrial dysfunction and the activation of apoptosis [38, 40]. HSP60 is also found in the cytosol, extracellular space, plasma membrane, and body fluids, where it performs non-canonical functions in inflammation, carcinogenesis, and immune responses [39]. Structurally, HSP60 forms a large, double-ring barrel-like complex, characterized by a closed-lid conformation that requires the co-chaperone HSP10 only in the presence of ATP [38], governing monomer interactions and ATP hydrolysis. This ATP-dependent structural change allows proper protein folding, after which the correctly folded protein is released into the cytoplasm [39].

2.6. HSP40

HSP40, a member of the DnaJ family, is distinguished by the presence of a J-domain and is classified into three types—type I, type II, and type III—based on its structural and functional traits. In both types I and II, the J-domain is located at the N-terminus, while the peptide-binding region is at the C-terminus. Conversely, type III HSP40 features a J-domain that can be positioned anywhere within the protein sequence. HSP40 recruits HSP70 via its J-domain to perform various functions [41]. HSP40's primary function is to regulate ATP-dependent polypeptide binding in HSP70. Additionally, HSP40 stabilizes HSP70 by facilitating the conversion of HSP70-ATP to HSP70-ADP, a process that depends on HSP40's J-domain [42].

Table 2. Types of sHSPs and their features.

Major Members	Nomenclature	Molecular Weight (kDa)	Stress inducibility	Tissue Distribution
HSP 27	HSP B1	22.8	Yes	Ubiquitous
MKBP	HSP B2	20.2	No	Muscle
HSP L27	HSP B3	17.0	No	Muscle
αA-crysallin	HSP B4	19.9	No	Eye lens
αB-crysallin	HSP B5	20.2	Yes	Ubiquitous
HSP 20	HSP B6	16.8	No	Muscle, brain
cvHSP	HSP B7	18.6	No	Muscle
HSP22	HSP B8	21.6	Cell type dependent	Muscle, brain
CT51	HSP B9	17.5	No	Testis
ODF1	HSP B10	28.3	No	Testis

Abbreviations: HSP: heat shock protein, MKBP: myotonic dystrophy kinase binding protein (HSPB2), CT51: cancer/testis antigen 51 (HSPB9), cvHSP: cardiovascular HSP, ODF1: outer dense fiber protein 1 (HSPB10).

2.7 Small HSPs

Small HSPs are a family of low-molecular-weight chaperones (12–43 kDa) encoded by 10 genes in humans (Table 2). They are categorized into two classes based on structure and expression: Class I sHSPs (e.g., HSPB1, HSPB5, HSPB6) contain a conserved α-crystallin domain and are heat-induced, phosphorylation-sensitive

chaperones that promote cell survival under stress. Class II sHSPs (e.g., HSPB8) lack this domain and are often involved in differentiation and tissue-specific functions. A key functional distinction is that sHSPs bind non-native proteins to prevent irreversible aggregation but, unlike ATP-dependent chaperones such as HSP70, do not promote refolding. Additionally, sHSPs exhibit broad anti-apoptotic activity across multiple cell death pathways [43, 44].

The primary structure of sHSPs consists of a structured α-crystallin domain (ACD), a flexible N-terminal region (NTR), and a short C-terminal region (CTR). The ACD consists of a compact sandwich structure made from two anti-parallel sheets of three- and four-strand DNA. The CTR plays a significant role in the oligomerization of sHSPs. The NTR enables oligomer formation and interacts with the substrate proteins [44]. Table 3 summarizes the functions of HSP families, including sHSPs.

Table 3. Functions of HSP family.

HSP Family	Key Members	Molecular Weight (kDa)	Cellular Location	Main Functions
Small HSPs (sHSPs)	HSPB1 (HSP27), HSPB5 (αB-crystallin), HSPB8	12–43	Cytosol, Nucleus, Mitochondria	ATP-independent chaperones; prevent protein aggregation; stress response
HSP40 (DNAJ)	DNAJA1, DNAJB1, DNAJC6	~40	Cytosol, ER, Mitochondria	Co-chaperone of HSP70; regulates ATPase activity; protein folding
HSP60	HSPD1 (HSP60)	~60	Mitochondria	ATP-dependent protein folding; folding of mitochondrial proteins
HSP70	HSPA1A (HSP72), HSPA8 (HSC70), HSPA5 (GRP78), HSPA9 (Mortalin)	~70	Cytosol, ER, Mitochondria	Major chaperone; protein folding, refolding, degradation; stress response
HSP90	HSPC1 (HSP90α), HSPC2 (HSP90β), HSPC4 (GRP94)	~90	Cytosol, ER	Protein folding, stabilization, signal transduction; client protein maturation
HSP100	HSP104 (yeast), ClpB (bacteria)	~100	Cytosol, Nucleus	Disaggregation of protein aggregates; thermotolerance
HSP110 and 170	HSP110, GRP170(HSP70 superfamily)	~110–170	Cytosol, ER	Chaperone activity; immunostimulatory; vaccine adjuvants

Abbreviations: HSP: heat shock protein, ER: endoplasmic reticulum, GRP: glucose-regulated protein.

2.7. Extracellular HSPs

HSPs are not only present intracellularly during stress to perform chaperone roles but can also be detected extracellularly, where they activate signaling pathways. These extracellular HSPs function as stress signals, alerting other cells, particularly immune cells, to prevent widespread damage.

Extracellular HSPs, particularly HSP70, can function as immunomodulatory signals, exhibiting either immunostimulatory or immunosuppressive effects depending on context [45]. Their release occurs via non-classical pathways, independent of the ER-Golgi system. Three primary mechanisms have been proposed: 1) the lysosome-endosome pathway, where HSP70 is transported into lysosomes but escapes degradation for extracellular release; 2) secretion via secretory-like granules; and 3) lipid-associated release, where HSP70 incorporates into lipid rafts and is exported in extracellular vesicles (EVs), such as exosomes. These HSP70-containing EVs are key mediators of intercellular communication and immune regulation [45-47].

3. Effects of HSPs on NDs

3.1. Aging and HSR

Aging is the principal risk factor for NDs, primarily driven by a progressive decline in proteostasis, gene mutations, and epigenetic changes [48]. Important to this collapse is the age-related attenuation of the HSR, characterized by reduced HSF1 levels and transcriptional activity, which has been consistently observed across species from Drosophila to non-human primates and humans [49]. This impaired HSR leads to diminished expression of cytoprotective chaperones, including HSP70, HSP90, and small HSPs, creating a permissive environment for protein misfolding and aggregation (Fig. 2). For instance, studies in vervet monkeys demonstrate that low skeletal muscle HSP70 levels predict progression of insulin resistance [50], while in Drosophila, specific small HSPs such as CG14207 and HSP67BC have been shown to extend lifespan through distinct mechanisms—HSP70-dependent refolding and HSP70-independent suppression of toxic aggregation, respectively [51].

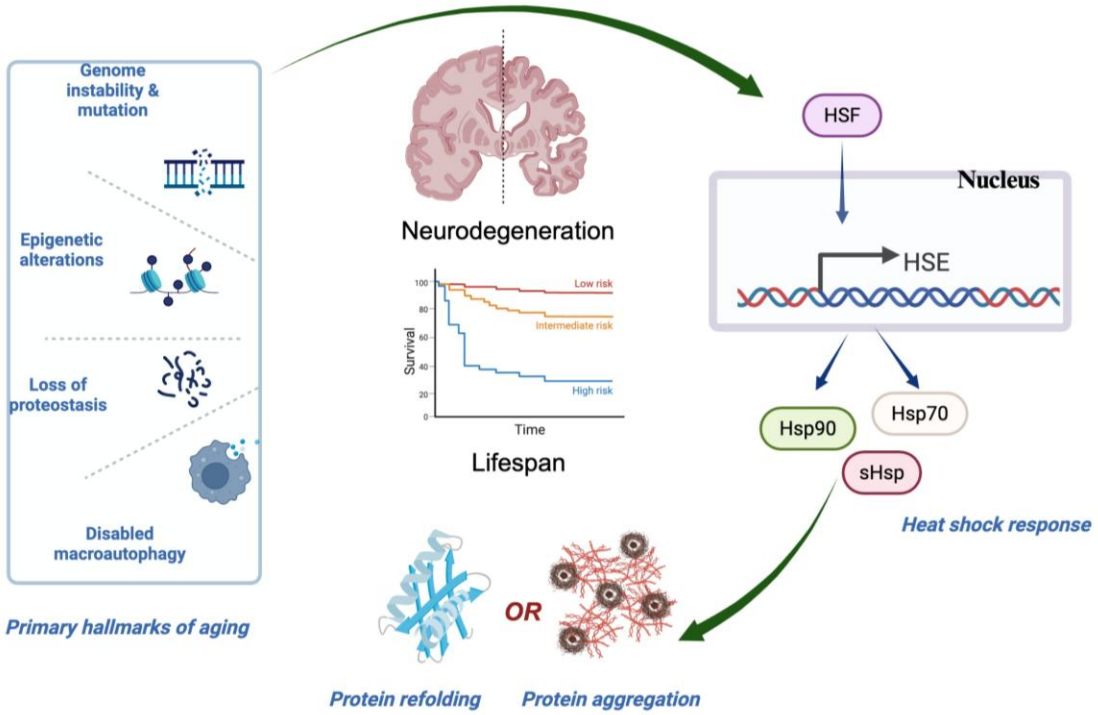

Figure 2. Heat Shock Proteins in Aging. Gene mutations, epigenetic drift, and progressive decline in proteostasis and autophagy—the core hallmarks of aging—initially activate HSF1 as a compensatory stress response, triggering transcriptional upregulation of cytoprotective chaperones such as HSP90, inducible HSP70 (HSPA1A), constitutive HSC70 (HSPA8), and sHSPs like HSPB1 and HSPB5. This acute HSR transiently restores protein folding capacity, suppresses aggregation of misfolded species, including Aβ, tau, and α-synuclein, and inhibits apoptotic cascades, thereby exerting beneficial effects on proteostasis and promoting neuronal resilience. However, chronic exposure to these aging stressors induces HSF1 desensitization through inhibitory post-translational modifications, epigenetic silencing of target genes, and persistent sequestration by overwhelming the capacity to misfold clients. This paradoxically diminishes HSP expression when most needed, while residual chaperone activity may inadvertently stabilize toxic oligomers or amplify neuroinflammatory signaling pathways. Consequently, this dual failure not only exacerbates proteostatic collapse but also creates a permissive environment for neuronal death, accelerating neurodegeneration and shortening lifespan, thereby illustrating that the net influence of HSF1-mediated HSP expression during aging critically depends on stress duration, cellular context, and the integrity of feedback control mechanisms.

This chaperone deficiency converges with and exacerbates age-related mitochondrial dysfunction [52], as evidenced by the role of mitochondrial HSP90 homolog TRAP1 in maintaining membrane potential and preventing mitochondrial permeability transition pore (mPTP) opening, thereby reducing ROS production [53]. The resulting oxidative stress further burdens the already compromised proteostasis network, fostering a vicious cycle of cellular decline [48]. Consequently, the aging brain becomes uniquely vulnerable to neurodegeneration, a vulnerability exacerbated by the age-associated decline in chaperone function.

3.2. HSP110

HSP110 doubles as a NEF and a client-binding co-chaperone that reactivates HSP70's disaggregase cycle. In Parkinson models, virally driven HSP110 (AAV9-Syn, 5×10^{11} vg) elevates nigral HSP70 30 %, halves phospho-α-syn inclusions, rescues 25 % of TH^+ neurons, and prolongs rotarod performance 20 % for at least 24 weeks [54, 55].

HSP110 modulates the aggregation of the amyloid beta (Aβ) peptide. In the C. elegans Aβ1-42 model, pan-neuronal HSP-110::GFP over-expression lowered Aβ1-42-mScarlet fluorescence lifetime by ≈0.5 ns, doubled Aβ aggregate load, and raised severely-damaged IL2 neurons from 20 % to 70 %; conversely, HSP-110 RNAi (≈55 % knock-down) extended Aβ lifetime by 0.3 ns and cut aggregation by 50 %, yet shortened median lifespan of Aβ animals by 2 days, revealing a narrow proteostatic window for HSP-110 [56]. Additionally, quantitative imaging of the double-tagged autophagy reporter LGG-1::mCherry::GFP in head neurons showed that HSP-110 RNAi raised the number of autophagosomes from 8 ± 3 to 15 ± 4 and autolysosomes from 5 ± 2 to 11 ± 3 per animal ($p \leq 0.0001$); the parallel decrease of the autophagy substrate SQST-1/p62 to 60 ± 8 % of control levels

confirms that HSP110 depletion enhances, rather than blocks, autophagic flux, providing a compensatory route for Aβ aggregate clearance when disaggregation is impaired [56].

3.3. HSP100

In yeast and human cells, engineered HSP104 (part of HSP100) systems can artificially redirect toxic aggregates, such as mutant huntingtin (mHtt), to non-traditional cellular locations, thereby reducing their cytotoxicity. Biophysically, HSP104 inhibits Aβ42 fibril formation at substoichiometric ratios by selectively binding with high affinity to sparsely populated, on-pathway aggregation nuclei, thus preventing their elongation into mature fibrils. HSP104's dual mechanisms of spatial sequestration and nucleation inhibition make it a promising therapeutic platform against neurodegenerative protein-misfolding disorders [57, 58].

3.4. HSP90

Inhibiting HSP90 releases HSF1, enabling it to move to the nucleus, and encourages the C-terminus of HSC70-interacting protein (CHIP)-mediated degradation of HSP90 client proteins by disrupting HSP90's chaperone cycle (Fig. 3). The HSP90 complex stabilizes proteins and sustains pathology-related changes that contribute to neuronal degeneration and dysfunction in neurodegenerative disorders [59].

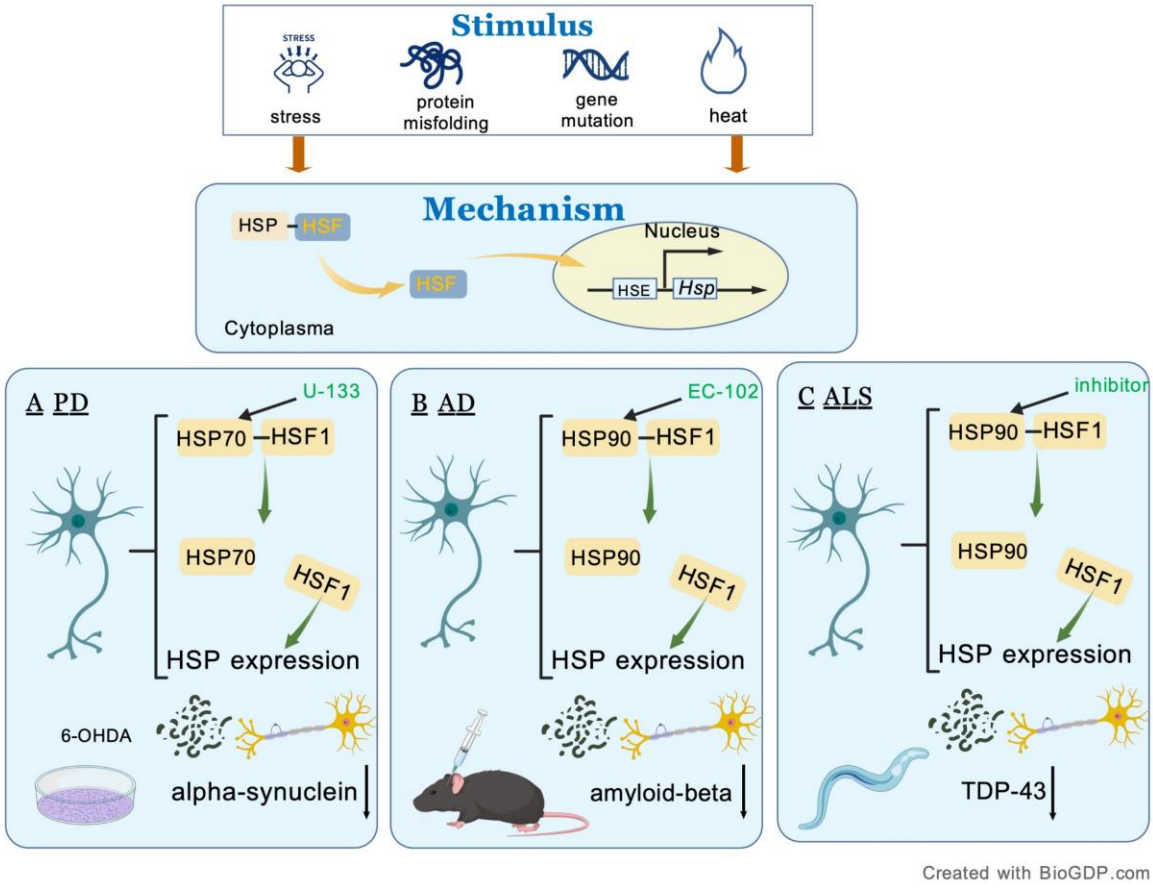

Figure 3. Heat-Shock Response in Neurodegeneration. Under proteotoxic stress—triggered by heat, environmental toxins, oxidative damage, or accumulation of misfolded amyloidogenic proteins—cytosolic HSP90 and HSP70 chaperones dissociate from their inhibitory complex with monomeric HSF1. This liberation enables HSF1 to undergo a conformational shift, trimerize, and translocate into the nucleus via importin-α/β pathways. Nuclear HSF1 trimers bind to conserved heat-shock elements (HSEs, nGAAn repeats) in promoter regions, driving rapid transcriptional upregulation of HSP70 isoforms (HSPA1A/HSC70), HSP90, HSP40 co-chaperones, and small HSPs. The resulting chaperone network directly engages disease-specific clients: HSP70/HSP40 complexes disaggregate α-synuclein oligomers in PD, while HSC70 mediates chaperone-mediated autophagy (CMA) of tau and Aβ in AD. In ALS, HSP90 stabilizes TDP-43, but its inhibition promotes CHIP-mediated ubiquitination and proteasomal clearance. This HSF1-dependent restoration of proteostasis is neuroprotective, yet age-related decline in HSF1 responsiveness, coupled with epigenetic silencing of HSEs and chronic chaperone sequestration, progressively blunts this response, accelerating neuronal vulnerability and disease progression and making HSF1 activation a compelling, stage-specific therapeutic target. Abbreviations: AD – Alzheimer's disease; ALS – Amyotrophic lateral sclerosis

Emerging evidence indicates that modulating HSP90 is a compelling therapeutic strategy for proteinopathies. A systemic 7-day treatment with EC102 in 13-14-month-old H-tau mice robustly reduced soluble phosphorylated tau (by ~75% at S396/S404 and ~50% at S202/T205) without altering total tau levels, concurrently elevating brain HSP70 by 1.6-fold and demonstrating high target engagement in the temporal cortex [60]. Conversely, in an amyloid model, intrathecal jujuboside A (5 mg kg^{-1} d^{-1}, 7 d) in APP/PS1 mice boosted HSP90β (~2-fold), concurrently reducing Aβ42 (45%) and plaques (35%), enhancing microglial phagocytosis (1.8-fold), and shortening Morris water maze escape latency from 28±3 s to 16±2 s, with all benefits abolished by Axl inhibition [61]. The clinical relevance of HSP90 is further underscored by its altered expression, alongside co-chaperones SGT1 and CHP-1, in dementia with Lewy bodies [62]. Beyond HSP90, other chaperones like HSP110 directly modulate Aβ aggregation and toxicity in C. elegans, with depletion proving protective [56, 60], highlighting the complex chaperone network as a rich therapeutic frontier.

Multiple chaperones, including HSP70, HSP90, sHSPs, and HSP104, counteract α-syn aggregation and toxicity. HSP90 plays a particularly complex role by stabilizing toxic α-syn oligomers in an ATP-independent manner, yet its inhibition also emerges as a therapeutic strategy. Inhibiting HSP90 promotes the degradation of key pathogenic proteins in PD, such as mutant LRRK2 and unstable PINK1, via ubiquitin-proteasome pathways. Brain-penetrant HSP90 inhibitors (e.g., PU-H71, XL888) rapidly reduce pathogenic LRRK2 levels by ≥50%, suppressing neurotoxic signaling and α-syn pathology. Similarly, HSP90 inhibition destabilizes PINK1, which depends on the HSP90/Cdc37 complex for stability. Furthermore, the co-chaperone p23 links HSP90 to the PHD2-HIF1α pathway, and its knockdown protects dopaminergic neurons, suggesting that p23 is another potential target. However, the therapeutic targeting of HSP90 requires caution due to concerns about specificity and off-target effects, underscoring the need for further optimized interventions [63-65].

HSP90 also interacts with mHtt, potentially stabilizing it. Although HSP90 overexpression did not improve Huntington disease (HD) phenotypes, RNAi-mediated silencing of HSP90 selectively lowered mHtt levels in neuronal cultures, implying mHtt is a client of the HSP90 chaperone complex. In the R6/2 mouse model, the brain-penetrant HSP90 inhibitor HSP990 dissociated HSP90 from HSF1, transiently up-regulating HSP70/HSP40 and reducing cortical mHtt aggregates by 20%. Thus, pharmacologic inhibition of HSP90 can suppress mHtt through both chaperone network activation and destabilization of its toxic client [66]. Pharmacological inhibition of HSP90 also transiently activates HSF1 and reduces mHtt aggregates in early-stage HD mice [67]. Also, NVP-AUY922 (HSP inhibitor) treatment substantially reduced soluble full-length mHtt levels in HdhQ150 embryonic stem (ES) cells and in ES cell-derived neurons[68]. HSP90 inhibition also decreases total and phosphorylated TDP-43 levels, increases HSP-16.1 expression, and protects against TDP-43 neurotoxicity in the C. elegans model [69].

3.5. HSP70

HSP70 isoforms play vital roles in protein folding and in preventing the buildup of toxic misfolded protein aggregates linked to NDs such as HD, AD, and PD (Table 4).

Table 4. HSP70 isoforms in neurodegeneration.

Isoform	Common Name	Primary Localization	Key Functional Roles	Association with Neurodegenerative Pathology
HSPA1A	HSP72, Inducible HSP70	Cytoplasm, Nucleus	Primary stress-responsive chaperone; prevents aggregation, promotes refolding, anti-apoptotic.	**PD:** Reduces α-synuclein aggregates, protects dopaminergic neurons, mitigates oxidative stress. **Tauopathies:** Binds tau, prevents aggregation, promotes solubility. **ALS:** Prevents TDP-43 and FUS aggregation.
HSPA8	HSC70, Constitutive HSP70	Cytoplasm	Core chaperone for CMA; constant "housekeeping" folding.	**HD:** Facilitates degradation of mHtt via CMA. **AD:** Implicated in CMA-dependent tau clearance.
HSPA5	BiP, GRP78	ER	ER stress sensor; key regulator of unfolded protein response (UPR); ER protein folding.	Implicated in AD and other diseases involving ER stress, though its specific role in the pathologies described above is less direct.
HSPA9	GRP75, mtHSP70	Mitochondrial matrix	Mitochondrial protein import and folding; mitochondrial quality control.	Linked to familial forms of PD (e.g., PINK1/Parkin pathway) through its role in mitochondrial homeostasis.

Abbreviations: HSP: heat shock protein, HSC: heat shock cognate 70 kDa protein, GRP: glucose-regulated protein, CMA: chaperone-mediated autophagy, ER: endoplasmic reticulum, PINK1: PTEN-induced kinase 1.

In PD, compensatory upregulation of the HSP70 family, including HSPA1A, along with HSP60 and HSP90, has been observed in the substantia nigra of Parkin-null mice, suggesting a protective adaptation to the loss of Parkin function [70]. Exogenously applied HSP70 also localizes to mitochondria, lysosomes, and the ER, where it enhances mitochondrial membrane potential, activates mitophagy, and protects against rotenone-induced toxicity in familial PD models [71]. Direct evidence supports the therapeutic potential of HSP70: its overexpression inhibits α-syn aggregation [72], rescues approximately 73% of dopaminergic neurons, markedly reduces oxidative stress (4.2-fold decrease in ROS) and apoptosis (11-fold reduction in cleaved caspase-3), and significantly improves motor performance and survival under oxidative stress [73]. Furthermore, exogenous HSP70 administration demonstrates broad neuroprotective efficacy. Intranasal delivery of HSP70 alleviates 6-OHDA-induced nigrostriatal damage by suppressing microglial activation and astrogliosis, and by modulating inflammatory cytokine expression [74]. Knockdown of HSP70 exacerbates lactacystin-induced dopaminergic neuron loss and motor deficits, whereas systemic administration of U-133, an HSF1 activator, potently induces HSP70 and its co-chaperone Hdj1. This induction halts TH^+ neuron death, preserves dopaminergic markers, reduces α-syn aggregates and microgliosis, and restores fine motor function in a rat PD model [75]. Pharmacological and genetic strategies to enhance HSP70 function consistently improve proteostasis. In SH-SY5Y cells, pretreatment with the HSP90 inhibitor 17-AAG (200 nM) increased HSPA1A levels 4-fold, restored proteasome activity to 85% of control, and enhanced autophagic flux (LC3-II doubled). Similarly, lentiviral-mediated HSP70 overexpression reduced ubiquitinated aggregates by 55% and maintained 80% cell viability under lactacystin challenge [76]. The efficacy of 17-AAG was further validated in Drosophila parkin mutants, where it improved climbing ability, restored dopamine levels, and ameliorated mitochondrial pathology [70, 76]. These findings collectively underscore HSP70 induction—achievable through small-molecule activators (e.g., MAL1-271), natural compounds (e.g., curcumin, glutamine), or physiological stressors (e.g., cold-water stress)—as a promising therapeutic strategy for PD [70].

In tauopathy, HSP70 exhibits a strong preference for pathological tau conformers, binding fibrils with high affinity (Kd ≈ 19 nM) and oligomers with moderate affinity (Kd ≈ 170 nM). This interaction reduces tau-induced membrane permeabilization by over 50%, thereby shielding cellular organelles from toxicity [77]. Functionally, elevating HSP70 levels via geldanamycin treatment redistributes tau from an insoluble, aggregated state to a soluble pool competent for microtubule binding, as demonstrated by a decrease in the insoluble tau fraction from 0.5% to 0.2% of total tau [78]. The therapeutic potential of this interaction is highlighted by the chemical probe JG-48, which stabilizes the HSP70-tau complex, doubles their binding affinity, and promotes a ~50% reduction in tau levels in cellular models [79]. Separately, HSP70 also demonstrates a capacity for liquid-liquid phase separation (LLPS), through which it chaperones fused in Sarcoma (FUS) in stress granules and suppresses its amyloid formation [80]. The essential role of HSP70 in proteostasis extends to TDP-43 proteinopathy, as implicated in ALS. Knockdown studies in human neuroblastoma cells show that HSP70 depletion impairs the refolding of misfolded TDP-43, leading to its toxic cytoplasmic accumulation and aggregation [59, 81].

Qi et al. demonstrated that HSC70 overexpression promotes the degradation of mHtt-552 by interacting with it and facilitating its transport into lysosomal lumens [82]. This process reduces overall mHtt accumulation. Conversely, when HSC70 is knocked down, Htt accumulation increases.

Enhancing HSP70 through a chemical compound mitigates behavioral defects in the chromosome 9 open reading frame 72 (C9orf72) mouse model and UBQLN2 can recognize HSP70 ubiquitination to facilitate poly-GA degradation [83]. T-cell intracellular antigen-1, upregulated in amyotrophic lateral sclerosis (ALS) patients and C9orf72-poly-GA mice motor cortex, mediates stress granule (SG) formation, sequestering HSP70 mRNA into SGs and reducing HSP70 expression, impairing poly-GA aggregate degradation via the UBQLN2-HSP70 pathway [84]. HSP70 and its co-chaperone HSP40 bind elongated polyQ tracts, sequestering monomers and dismantling spherical/annular oligomers; parallel cell assays suggest similar suppression of polyalanine aggregation, though direct proof remains sparse [85-87].

3.6. HSP60

HSP60 haploinsufficiency of *HSPd1*, the gene encoding HSP60, triggers a multi-layered pathogenic cascade that mirrors spastic paraplegia 13 (SPG13). Magnoni et al. established that reduced HSP60 expression compromises mitochondrial structural integrity and respiratory chain function, directly impairing neuronal viability [88]. This mitochondrial dysfunction extends to proteomic destabilization: HSP60 deficiency disrupts the mitochondrial matrix proteome, depleting critical protein levels and impairing cholesterol biosynthesis. This molecular defect mechanistically accounts for the hypomyelination phenotype observed in both cellular models and zebrafish carrying HSP60 variants [89]. Compounding these defects, HSP60 silencing initiates a

detrimental signaling feedback loop: increased ROS production activates AMPK while concurrently suppressing mTORC1 signaling, culminating in global suppression of protein translation and arrested cell growth [90].

Marino et al. demonstrated that the HSP60 directly binds to Aβ oligomers (Aβo) at a 25:1 molar ratio, reducing their cytotoxicity by 35% in SH-SY5Y cells. HSP60 pretreatment also significantly rescued Aβo-impaired long-term potentiation (LTP) in mouse hippocampal slices and decreased Aβo binding to synaptosomes by ~68% [91]. Another study focusing on Aβ fibrillogenesis in AD demonstrated that the direct interactions between HSP60 and Aβ peptides, both in the mitochondria and the extracellular space, affect Aβ amyloid aggregation. Chaperonin HSP60 completely inhibits the formation of β-sheet-rich amyloid aggregates by blocking the molecular pathway [92].

Zhao et al. demonstrated that transplanting hUC-MSC-derived dopaminergic-like neurons into 6-OHDA-lesioned PD rats significantly reduced apomorphine-induced rotation. This recovery was accompanied by a marked increase in HSP60 protein levels, up to 153.33% in the striatum at 6 weeks post-grafting [93].

3.7. HSP40

HSP40 plays a crucial role in regulating HSP70 chaperone activity. Minami et al. concluded that HSP40 facilitates the hydrolysis of HSP70, increasing its steady-state ATPase activity by approximately 7-fold [94]. Through multivalent interactions, DNAJB1 recognizes the oligomeric form of α-syn fibrils at high concentrations, enabling HSP70 to perform its chaperone function on the aggregates. The same study also found that DNAJB1 promotes the accumulation of HSP70 on the amyloid surface, organizing it on the fibril surface and boosting its affinity for more effective amyloid disaggregation [95]. Indeed, HSP40 (DNAJ) co-chaperones specifically recognize and bind to exposed hydrophobic segments of unfolded or misfolded polypeptide substrates, stimulating the ATPase activity of HSC70 and promoting ATP hydrolysis to ADP. This mechanism is essential for maintaining cellular protein homeostasis [96]. Overexpressing HSP40 chaperones, such as DNAJB1, in HEK293T cells can alleviate toxicity associated with FUS aggregates and even TDP-43 toxicity in rodent neurons. This is achieved by restoring the inhibited ubiquitin-proteasome system caused by TDP-43 or FUS [97].

3.8. Small HSPs

In PD and dementia with Lewy bodies, sHSPs exhibit protective functions against α-syn. Research has highlighted the roles of HSP27 and αB-crysallin in reducing the number of cells containing α-syn inclusions, demonstrating their inhibitory potential. Notably, these sHSPs can interact with small α oligomers, preventing their progression to fibrillar species [98]. Bruinsma et al. identified that, beyond HSP27 and αB-crysallin, additional sHSP members, including HSP20, HSPB8, HSPB2, and HSPB3, also participate in regulating α-syn aggregation [46]. HSP10 interacts with HSP60 in a nucleotide-dependent manner, forming distinct complexes that influence protein folding efficiency [99]. In experimental models, HSPB8 overexpression reduced mutant superoxide dismutase 1 (SOD1) aggregates by approximately 5–6 fold. HSPB8 also nearly eliminated insoluble oligomers of truncated TDP-43, highlighting its strong anti-aggregation role [100].

In AD, HSPB5 and HSPB8 colocalize with β senile plaques and are upregulated in the brain [101], whereas increased HSPB5 expression can alleviate these symptoms [102]. Multiple sHSPs, including HSPB1, HSPB5, HSPB6, and HSPB8, inhibit the oligomerization of Aβ monomers and prevent oligomers from progressing into fibrils [103, 104]. Furthermore, HSPB1 demonstrates partial protective effects against tauopathy, another hallmark of AD [105], highlighting its therapeutic potential.

3.9. Extracellular HSPs

In AD, extracellular HSP70 binds Aβ with high affinity, which may help prevent disruption of neuronal cell membranes and the formation of cytotoxic large oligomeric Aβ species [47, 104, 106]. Furthermore, the presence of extracellular HSP70 may reduce the Aβ's interaction with synaptic membrane proteins, potentially enhancing synaptic transmission [107, 108].

In ALS, administering HSP70 improves symptoms and slows disease progression. In G93A-SOD1 ALS mice, thrice-weekly i.p. injections of 20 μg rhHSP70 starting from P30 preserved 19–21 μm large-caliber ventral-root axons, maintained about 80% innervation of neuromuscular junctions in medial gastrocnemius and tibialis anterior muscles at P75, and delayed the onset of hind-limb tremors by 11 days compared to untreated mice; lifespan increased from 128 to 137 days. Lumbar motoneurons (MNs) retained a soma area of 162 μm^2 at P120, while microglial Iba1 fluorescence decreased by 50% [109, 110]. Furthermore, the administered HSP70 was not detected in the mice's spinal cords. Still, it was found in the muscle, suggesting that HSP70 may promote survival through peripheral mechanisms by preserving the MNs that innervate skeletal muscles[110].

4. HSP Mutations in NDs

4.1. HSP110

Mutations in *HSP110* can affect its expression, leading to tauopathies such as AD. HSP110 deficiency causes the buildup of hyperphosphorylated tau and the formation of neurofibrillary tangles (NFTs), which are involved in the development of NDs [111]. A key cause of tau pathology is reduced peptidyl-prolyl isomerase (PPIase) activity, an enzyme vital for clearing and dephosphorylating tau. PPIases, especially Pin1, help dephosphorylate tau by activating protein phosphatase 2A (PP2A), a major regulator of tau balance. Losing *HSP110* impairs both PPIase and PP2A functions, worsening tau problems [112]. Moreover, the absence of HSP110 also promotes the buildup of Aβ plaques by interfering with the processing of the amyloid precursor protein (APP). This boosts amyloidogenic pathways, resulting in the early formation of Aβ plaques [113].

4.2. HSP90

HSP90 is typically present in large quantities, acting as a protein-folding "buffer" that supports cell growth and various cellular functions of different proteins[114]. Mutations in both *HSP90* and its co-chaperones can lead to multiple human diseases.

There are about 50 different cochaperones that collaborate with HSP90 to regulate protein folding. Mutations in about 20 cochaperones have been identified, resulting in various phenotypes that affect brain, muscle, eye, and heart function. *DNAJC7* is a highly constrained gene (pLI=0.99) encoding an HSP40 co-chaperone with both J-domain and tetratricopeptide repeat motifs. Farhan et al. identified six distinct protein-truncating variants (PTVs) in *DNAJC7* in ALS patients, with significant enrichment in cases (e.g., 8 carriers among 5,095 cases vs. zero among 28,910 controls), supporting its role as a novel ALS risk gene [115]. Mutations in other co-chaperones, such as cytochrome b5 reductase 4, FK506 binding protein 6, and translocase of the outer mitochondrial membrane 70 homolog A, can lead to the development of NDs [116].

Missense mutations in *HSP90α* (*E381K, T525I*) or its co-chaperone *CDC37* (*R6G, V218M*) cut ATPase activity 35–60 %, destabilizing 217 neuronal kinases and lowering cortical HSP90 28 %. The same lesions simultaneously hyperactivate 84 oncogenic kinases, including BRAF and CDK4, driving glioblastoma and paraneoplastic syndromes that ectopically secrete neurotoxic peptides. Consequently, carriers exhibit both earlier PD onset (52 ± 4 vs 61 ± 5 years) and accelerated AD progression (CDR 1→2 in 1.8 vs 3.1 years), while 32 % of cancer patients develop cognitive or motor deficits within 3 years [117-119].

4.3. HSP70

A mutation in the *HSP70* gene can lead to diseases like neurodegenerative disorders and colorectal cancer. This mutation may occur at different sites, including the NBD and SBD. Mutations in these areas interfere with HSP70's chaperone function. However, these mutations are less frequent and might not be the sole cause of NDs [120].

In addition, polymorphisms in *HSP70* genes are associated with PD susceptibility. In the *HSP70-1* gene, the -110 A/C and +190 G/C polymorphisms are associated with an increased risk of PD (OR 2.91, 95% CI 1.51-5.96). The -100 A/C polymorphism, which is crucial for heat-induced expression of the *HSP70-1* gene, is located three base pairs upstream of the HSE and is believed to alter *HSP70-1* gene regulation [121].

4.4. HSP60

Mutations in *HSP60* can lead to several NDs, notably SPG13, a form of hereditary spastic paraplegia, and mitochondrial HSP60 chaperonopathy (MitCHAP-60), an early-onset neurodegenerative disorder [122]. The *V72I* mutation leads to SPG13, while a missense mutation in *D3G* causes MitCHAP-60 [123]. These two mutations significantly reduce HSP60's chaperone-folding activity by decreasing ATP hydrolysis. Wang et al. found that *V72I* and *D3G* point mutations impair HSP60's ability to fold mitochondrial proteins, with the *D3G* mutation leading to more severe symptoms than the *V72I* mutation [122]. When treating the mutated HSP60 with ATP or ADP, it fails to form a stable complex and instead breaks into monomers. HSP60 deficiency directly impairs oxidative phosphorylation, reducing ATP production. This energy shortfall most severely impacts tissues with high metabolic demands, such as muscle and the nervous system, explaining their particular vulnerability in HSP60-related disorders [124].

4.5. HSP40

Mutations in *HSP40* can significantly disrupt its interaction with HSP70, particularly at the J-domain. A point mutation in the conserved His-Pro-Asp (HPD) motif within the J-domain of *HSP40* significantly decreases HSP40-HSP70 interactions [125]. The interaction between HSP40 (J-domain proteins) and HSP70 is critically dependent on a highly conserved HPD tripeptide motif (residues 32-34 in many HSP40s). This motif is essential for stimulating HSP70's ATPase activity. Substitution of the central histidine (H) with

glutamine (Q) disrupts this functional interface, abrogating the HSP40-HSP70 interaction. As a result, the mutant HSP40 fails to activate HSP70's ATP hydrolysis, leading to a severe impairment of the chaperone cycle and a consequent loss of HSP70-mediated protein refolding activity [30, 126].

Mutations in *HSP40* are associated with PD, Charcot-Marie-Tooth (CMT) disease, distal hereditary motor neuropathy, and cerebellar ataxia. These mutations can occur in various *DNAJ* genes. For instance, a cysteine-to-tyrosine substitution caused by a missense mutation in *DNAJB2* leads to distal hereditary motor neuropathies. Additionally, a mutation in *DNAJB6* causes limb-girdle muscular dystrophy type 1 [127].

4.6. Small HSP

A dominant missense mutation in *HSP27 (HSPB1)* is linked to CMT and distal hereditary motor neuropathy [128]. A mutation in the α-crystallin domain of *HSP27* causes hyperactivation, enhancing its protein-binding abilities by increasing monomerization, which affects microtubule remodeling and stability. These processes are essential for neuronal function, and their disruption can lead to neurodegenerative outcomes [129]. Mutations in *HSP22 (HSPB8)* are associated with distal myopathy and distal hereditary motor neuropathy: A frameshift mutation in the C-terminal region of *HSP22* results in a 50% reduction in HSP22 protein levels in the patient's muscles; alterations in HSP22 disrupt CMA, potentially increasing protein aggregation and contributing to disease pathology [130].

5. Gap in the Literature

5.1. Common Facts

Current reviews on HSPs in NDs mainly emphasize their vital role in maintaining proteostasis by preventing the aggregation of misfolded proteins—such as Aβ, tau, α-syn, and mHtt—through chaperone activities including refolding, sequestration, and facilitating degradation. They thoroughly discuss the cooperative functions of key HSP families: HSP70 systems (assisted by HSP40 and HSP110) in substrate binding and disaggregation, small HSPs in ATP-independent prevention of oligomerization, and specialized disaggregases like HSP104. Additionally, reviews highlight therapeutic strategies aimed at increasing HSP levels—such as HSF1 activators like Arimoclomol—or delivering engineered chaperones, while also addressing challenges in achieving target specificity and effective delivery to the CNS.

5.2. Negative Roles of HSPs

While traditionally viewed as protective, the chaperone system can paradoxically worsen the pathology of Aβ, tau, and α-syn. Certain HSPs can stabilize toxic soluble oligomers, preventing their removal and prolonging their harmful effects. For example, HSP70 and HSP27 can bind to oligomeric forms of Aβ and α-syn, unintentionally shielding them from degradation and promoting their spread of toxicity [128]. Additionally, HSP90 plays a key role in stabilizing hyperphosphorylated tau, blocking its breakdown and promoting its buildup and aggregation [129, 130]. In severe cases, as seen with the yeast disaggregase HSP104, dysfunctional activity can break down large aggregates into many smaller, seeding-competent species that increase pathology. Surprisingly, HSP110 overexpression may worsen Aβ pathology. Overstabilizing Aβ oligomers or disrupting the coordinated HSP70 chaperone cycle can hinder adequate clearance and increase neuronal damage, revealing a complex role in protein balance [51, 131]. Therefore, prolonged HSP activity in a failing proteostasis network can shift from a protective response to a factor that accelerates disease progression.

5.3. Rethinking HSP

Numerous excellent reviews have highlighted the essential role of HSP dysregulation in NDs, especially emphasizing protein aggregation in AD and PD [131, 132]. However, a comprehensive synthesis of emerging areas is missing: the direct connection between heritable HSP gene mutations and specific NDs; the effects of extracellular HSP signaling on neuroinflammation; and a thorough assessment of clinically translatable therapeutic strategies across different disease stages. This review aims to address these gaps by offering an integrated analysis of HSP genetics, non-cell-autonomous functions, and stage-specific therapeutic approaches, including advanced delivery platforms.

6. Diagnostic and Prognostic Biomarkers

HSPs have become promising biomarkers across different diseases, detectable in various forms such as serum, exosomes, and circulating lymphocytes. Elevated serum HSP70 levels correlate with tumor size in non-small cell lung cancer. Conversely, exosomal HSP70 and HSP90 from head and neck squamous cell carcinoma are linked to poor prognosis[133]. Here, HSP expression levels, their functional capabilities, and especially their sequestration within pathological protein aggregates can indicate cellular stress and proteostatic failure [134]. The presence of specific HSPs, such as DNAJB6, in biofluids, or their

co-localization with aggregates, such as α-syn or tau, in exosomes, offers potential for diagnosing and monitoring disease progression, reflecting a common principle of HSPs as biomarkers of proteotoxic stress in both cancer and neurodegeneration [135, 136].

6.1. Clinical Study

Based on computational analysis of brain tissue gene expression data, HSPA1A, HSPA2, and HSPA8, members of the HSP70 family, are identified as promising prognostic molecular biomarkers for AD [137]. The study found HSPA1A and HSPA2 were significantly upregulated in AD samples, while HSPA8 was downregulated. Critically, the combination of these three genes demonstrated a high diagnostic power for distinguishing AD from controls, with an impressive area under the curve (AUC) of 0.905. The hub genes also showed significant correlations with key AD-related genes (such as *APP, PSEN1/2, APOE*), β- and γ-secretase activities, and specific immune cell infiltration, highlighting their close involvement in AD pathogenesis and the immune microenvironment [138].

6.2. Challenges

Although HSPs have therapeutic potential, they face significant challenges as reliable biomarkers in NDs. Their main limitation is a lack of disease specificity, as levels can be raised by various stressors, including systemic inflammation [139].

More fundamentally, the link between peripheral blood levels and CNS pathophysiology remains uncertain due to four key mechanisms. First, the blood-brain barrier (BBB) significantly limits HSP exchange, with minimal physiological transport from brain to blood, and disease-specific dynamics where BBB breakdown occurs relatively late in AD progression (mid-stage rather than preclinical), creating a timing mismatch between neuronal stress and peripheral detection [140, 141]. Second, the cellular origin of blood-based HSPs is unclear, complicated by substantial peripheral contamination—especially platelet-derived HSP70 released during clotting that contaminates serum samples and masks CNS-specific signals [142, 143]. Third, their dynamic expression produces complex, non-linear patterns that are hard to interpret; brain HSP70 may shift from compensatory increases to exhaustion in late stages, showing biphasic kinetics where it peaks before symptoms appear then declines as neurons die, while serum levels paradoxically increase due to systemic inflammation and gliosis, highlighting the need for longitudinal monitoring rather than single measurements [144]. Fourth, different HSP isoforms within the same family, such as HSPA1A and HSPA8 in Alzheimer's, can show opposing expression patterns, requiring precise distinction and ratio-based analysis rather than just absolute quantification [137].

These biological challenges are worsened by a lack of standardized detection assays across laboratories [145]; pre-analytical variables such as serum versus plasma collection cause significant differences in HSP70 levels due to platelet release, and most commercial assays cannot distinguish isoforms or enrich CNS-derived exosomes without neuron-specific markers like L1CAM [146]. Therefore, clinical translation requires a multi-tiered validation approach: correlation with CSF levels, longitudinal monitoring with neuroimaging, and ultimately post-mortem brain tissue confirmation to determine whether blood signals truly reflect CNS proteostatic failure.

7. Therapeutic Potential for NDs

Several therapeutic drugs approved by the Food and Drug Administration (FDA) induce HSP activity. The therapeutic landscape for NDs is rapidly expanding, including various strategies targeting protein homeostasis and drug delivery. Several compounds have received FDA approval for related conditions, such as the chemical chaperones 4-phenylbutyric acid (4-PBA) [147], tauroursodeoxycholic acid (TUDCA) [148], and Arimoclomol [149]. Beyond pharmaceuticals, non-invasive approaches like heat therapy are being studied to activate the HSF pathway [150]. Additionally, new targets, such as CMA [151], are actively being explored to enhance the clearance of misfolded proteins.

Concurrently, advanced delivery systems are critical for translating these strategies into effective CNS therapies. Intranasal administration is widely studied for its potential to deliver drugs directly to the brain, bypassing the blood-brain barrier [152]. Furthermore, gene therapy utilizing adeno-associated virus (AAV) vectors [153] and specific promoters is gaining significant momentum as a promising modality for treating a range of human NDs (Fig. 4).

7.1. Chemical Chaperone

As an FDA-approved drug for urea cycle disorders, 4-PBA functions as a molecular chaperone that improves ER proteostasis and influences mitochondrial function [147, 154]. In HD models, 4-PBA treatment improved the neurodegenerative phenotype, extended lifespan, and enhanced motor function. These benefits are attributed to its dual role as a chemical chaperone that reduces protein aggregation and as a histone deacetylase (HDAC) inhibitor that promotes neuroprotective gene expression

[155, 156]. Similarly, in PD contexts, 4-PBA alleviates motor deficits and protects dopaminergic neurons by targeting mitochondrial dysfunction and astrocyte activation [157]. A significant challenge for its CNS application, however, is the impractically high dosage required for efficacy in preclinical models (0.5–20 g/kg/day) [154]. To overcome this, researchers have developed novel 4-PBA derivatives (IPA-1 and DCA-1) with superior binding affinities and pharmacokinetic profiles, showing promise for AD [158].

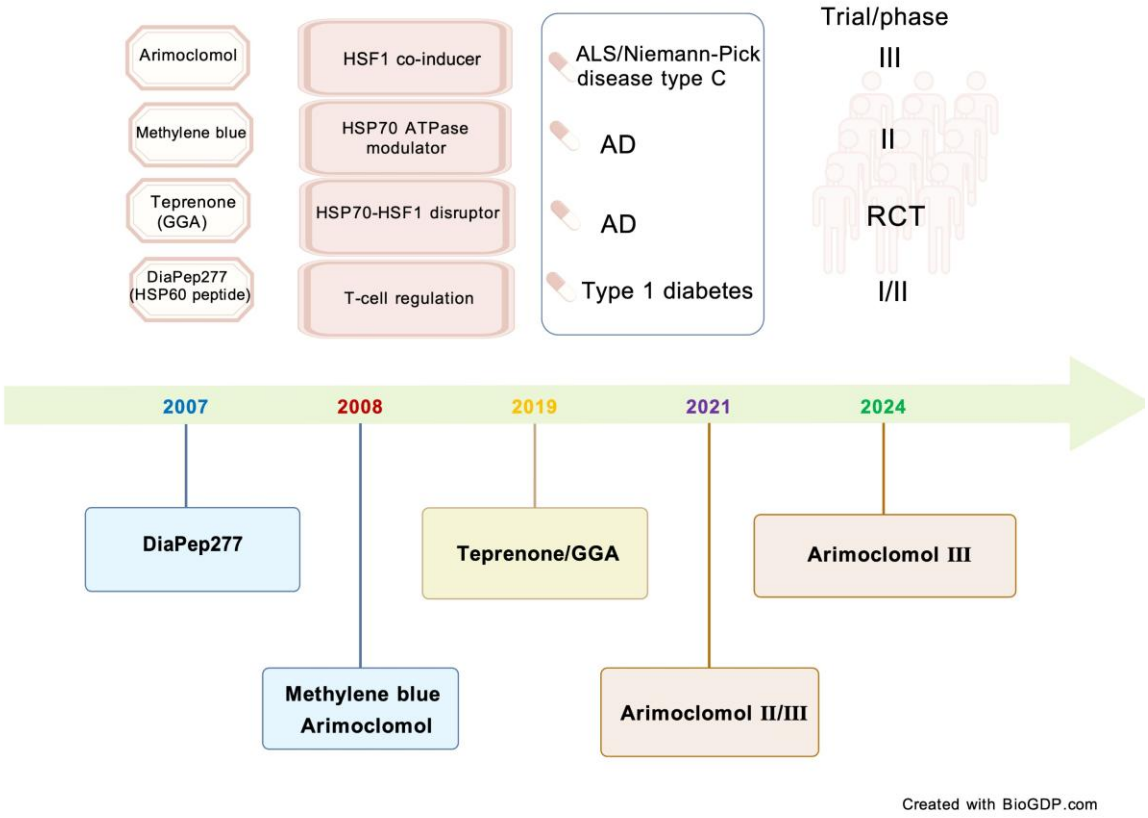

Figure 4. Timeline of HSP-Targeting Drugs in Neurodegenerative Diseases. The HSP-targeting timeline encapsulates both the therapeutic promise and translational pitfalls of modulating the proteostasis network across neurodegenerative diseases. Early momentum emerged from symptomatic HD and PD models, where HSP90 inhibitors and HSF1 co-inducers reduced mutant huntingtin and α-synuclein aggregation, respectively. This preclinical success catalyzed advancement into randomized clinical trials for AD, ALS, and NPC. Arimoclomol, an HSF1 co-inducer, achieved the first robust phase II/III success in NPC, demonstrating a statistically significant 65% reduction in annual disease progression by enhancing lysosomal activity and the HSR, though phase III trials in ALS failed due to poor tolerability and lack of efficacy at doses up to 1200 mg/day. Methylene blue, an HSP70 ATPase modulator, progressed to Phase III trials for AD after Phase II data showed 81% slowing of cognitive decline by locking HSP70 in a high-affinity ADP-bound state, facilitating tau clearance. Similarly, geranylgeranylacetone (teprenone), an anti-ulcer drug repurposed as an HSR inducer, provided early cognitive benefits in mild AD by crossing the blood-brain barrier and upregulating HSP70. Despite these advances, dose-limiting toxicities—hepatotoxicity and oncogenic risks from HSP90 inhibitors, gastrointestinal side effects from high-dose 4-PBA—and mixed efficacy outcomes remain formidable hurdles, underscoring the need for stage-specific interventions and biomarker-driven patient stratification. Abbreviations: AD – Alzheimer's disease, ALS – Amyotrophic lateral sclerosis, HD – Huntington's disease, NPC – Niemann-Pick disease type C.

TUDCA, an FDA-approved bile acid for liver disease, exhibits multiple neuroprotective mechanisms. It stabilizes mitochondrial membranes to inhibit apoptosis, reduces ER stress, and diminishes oxidative damage [159]. Studies in PD models demonstrate that TUDCA pretreatment protects dopaminergic neurons, suppresses neuroinflammation, and mitigates oxidative stress and autophagic dysregulation [160]. Its favorable oral bioavailability, low toxicity, and ability to cross the BBB make it particularly attractive for neurodegenerative applications [155]. However, its efficacy as a direct anti-aggregant is not universal. Notably, in vitro studies indicate that TUDCA fails to inhibit the oligomerization or fibrillization of Aβ peptides despite its potent cell-

protective effects [161]. This suggests its primary benefits may stem from enhancing cellular resilience rather than directly disrupting specific aggregation pathways.

7.2. Heat Shock Inducer and HSF1

In NDs, HSF1 levels are significantly reduced, leading to decreased expression of HSF1 target genes. It is important to note that as one ages, the level of HSF1 protein decreases [9], making HSF1 a promising therapeutic target, as most NDs develop with age. Various pharmacological molecules can activate HSF1 [162-164]. These activators can either inhibit HSPs that limit HSF1 activity or promote proteotoxic stress; the latter may not be an ideal approach for countering NDs, as it can increase misfolded and aggregated proteins, leading to cell death [165].

7.2.1. Pharmacological Molecules

Arimoclomol, an FDA-approved hydroxylamine derivative, acts as a co-inducer of HSP expression. It plays a role in amplifying HSP expression by stabilising the active, phosphorylated trimer form of HSF1 and extending the duration of HSF1's binding to the HSE. This process enhances the encoding of multiple major HSPs in response to cell stress. Treatment with Arimoclomol upregulates several HSPs, including HSP60, HSP70, HSP90, and GRP94. The therapeutic potential of Arimoclomol has been demonstrated in animal models with ALS [166]. Studies have shown that Arimoclomol exhibits beneficial effects as an anti-aggregation drug, rescues motor neurons, improves neuromuscular function, and extends lifespan when treatment is initiated at the onset of symptoms [167]. The Arimoclomol nanomicelles significantly decreased Aβ and α-syn aggregation and demonstrated anti-inflammatory effects in an acute inflammation model [168].

Geranylgeranylacetone (GGA), also known as Teprenone, is an anti-ulcer drug used in Japan. It induces HSR by disrupting the interaction between HSP70 and HSF1 through its binding to HSP70's SBD. Therapeutic intervention with GGA in diseased AD mice effectively reduced Aβ aggregation and synaptic loss, leading to recovery of cognitive abilities. This supports the drug repurposing of GGA, an already approved anti-ulcer medication, for AD [169]. Furthermore, GGA can cross the BBB, as these effects occurred during oral administration, making it an ideal candidate for NDs [169]. A randomized, double-blind, placebo-controlled study evaluated the efficacy of Teprenone in patients with mild-to-moderate AD. It showed that Teprenone significantly improved Mini-Mental State Examination scores, particularly in patients with mild medial temporal atrophy, suggesting potential effectiveness when administered before significant atrophy progression [170].

7.2.2. HSP90 Inhibitor

Geldanamycin (GA), a natural benzoquinone ansamycin, acts as a potent HSP90 inhibitor by binding to its N-terminal ATP-binding pocket and suppressing its ATPase activity. This inhibition triggers a compensatory stress response: the release and activation of HSF1, which upregulates the expression of downstream chaperones, including HSP70. The therapeutic potential of GA in NDs is supported by multiple studies [76, 165, 171, 172]. For instance, in a PD model, pretreatment with 200 nM GA reduced the number of cells bearing α-syn-positive inclusions by approximately 50%. It lowered total α-syn levels by about 75%, thereby preventing α-syn-induced toxicity. These protective effects are primarily attributed to GA-mediated induction of HSP70 [76]. However, the efficacy of GA is highly dependent on the disease stage. Evidence suggests that GA mainly prevents, rather than reverses, α-syn aggregation. Consequently, it is most effective during initial aggregate formation, making the early symptomatic phase a potential critical window for treatment [76].

7.2.3. Multitarget Molecular ASS234

The multitarget molecule N-((5-(3-(1-benzylpiperidin-4-yl) propoxy)-1-methyl-1H-indol-2-yl)methyl)-N-methyl prop-2-yn-1-amine (ASS234) exhibits potential for therapy that can counter NDs by enhancing the transcriptional activity of HSF1 and inducing various classes of HSPs [173]. ASS234 has attractive characteristics, including a low toxicity profile, no mutagenic concerns, and the ability to cross the BBB [174].

Ramos et al. showed that ASS234 induces HSF1 expression and its downstream HSP genes in SH-SY5Y cells [173]. This upregulation enhances the cell's capacity to degrade or refold misfolded polypeptides, a process critical for counteracting neurodegeneration. Furthermore, ASS234 exhibits significant anti-inflammatory properties, downregulating pro-inflammatory mediators (IL-6, IL-1β, TNF-α, TNFR1, NF-κB) while promoting anti-inflammatory genes (IL-10 and TGF-β) [175]. It also confers resilience against diverse cellular stressors, including oxidative stress, hyperphosphorylated tau, and Aβ-induced toxicity, without exhibiting cellular harm. These mechanistic insights translate into therapeutic benefits in vivo. In a mouse model of AD, ASS234 reduced Aβ aggregation

and neuroinflammation, ameliorated memory impairment, decreased amyloid plaque burden, and attenuated gliosis in the cortex and hippocampus [176].

7.2.4. Inducing HSF1 by Targeting Post-Translational Modification

HSF1 can be indirectly activated through regulation of post-translational modifications, such as acetylation, sumoylation, and phosphorylation. The acetylation and deacetylation of HSF1 can regulate the expression of chaperone proteins. Cyclic AMP-responsive element-binding protein and histone acetyltransferase p300 mediate the acetylation of HSF1 at lysine 80, leading to its dissociation from DNA and reducing the HSR [49, 177]. The SIRT1 activator resveratrol exerts neuroprotection by deacetylating key substrates (e.g., p53). In mammalian neurons, resveratrol (250 nM) reduced p25 and mutant SOD1-induced cytotoxicity by approximately 50% and 46%, respectively, rescuing polyQ-impaired neuronal function [165, 178].

Sumoylation, mediated by enzymes including E1 small ubiquitin-related modifier (SUMO) activating enzymes, can inactivate HSF1. SUMOylation acts as a negative regulator of HSF1 transactivation capacity. Pharmacological inhibition of the SUMO pathway using anacardic acid or ginkgolic acid (a component of *Ginkgo biloba* extract EGb 761) suppresses HSF1 SUMOylation, thereby enhancing its DNA-binding activity and potentiating the HSR [179, 180]. In a study conducted by Fukuda et al., ginkgolic acid was shown to inhibit sumoylation by preventing the formation of E1-SUMO intermediates through interactions with E1 enzymes, thereby inhibiting the conjugation of SUMO to target proteins [179]. Ginkgolic acid protects against Aβ-induced synaptic dysfunction in the hippocampus by enhancing LTP, restoring paired-pulse facilitation, and reversing Aβ-mediated effects on excitatory transmission [181]. Ginkgolic acid also promotes autophagy-dependent clearance of preformed α-syn aggregates in PD cell models, reducing Lewy body-like inclusions and enhancing neuronal survival without significant cytotoxicity [182].

Phosphorylation of serine 303 and 307 inactivates HSF1 and suppresses chaperone protein expression. A potential intervention involves inhibiting glycogen synthase kinase 3 (GSK3) to prevent phosphorylation of serine 303. GSK3 is a key therapeutic target in AD, with roles in tau pathology, amyloidogenesis, neuro-inflammation, oxidative stress, and mitochondrial dysfunction [183]. GSK3 inhibitors like SB-216763 or lithium can enhance HSF1's DNA binding and increase HSP70 expression. Besides protecting against cell death caused by protein aggregation, such as polyQ, GSK3 inhibition can also activate autophagy, making it an attractive therapeutic approach [165].

7.3. Peptides Derived from Heat Shock Proteins

Peptides derived from HSPs show promising potential for neuroprotection. These peptides can originate from various HSP sequences and regions and act as chaperones, promoting proper protein folding to prevent aggregation and neurotoxicity. Examples include shepherdin from HSP90, P277 resembling HSP60, and peptides from sHSP.

Functioning as an ATP-competitive inhibitor, the peptide shepherdin—derived from the survivin-HSP90 complex—binds to HSP90 and promotes the degradation of its client proteins[184]. This mechanism effectively targets misfolded and aggregated proteins, such as tau, underlying its potential not only in cancer but also in neurodegenerative disorders like PD by restoring proteostasis [78, 185].

P277 is a peptide derived from sequence 437-460 of HSP60, consisting of 24 amino acids, with two cysteine residues replaced by valines in the native sequence. P277 has been studied as a treatment for autoimmune diseases, including type 1 diabetes[186, 187]. Research has shown that P277 can modulate the immune response by downregulating spontaneous T-cell reactivity, thereby exerting immunomodulatory effects that can alter autoimmune responses [188]. In NDs such as AD, PD, HD, and ALS, inflammation caused by protein aggregates plays a crucial role in neurodegeneration[189]. Given P277's ability to modulate T-cell responses and reduce inflammation, it holds potential as a therapeutic strategy to regulate immunity and mitigate neuroinflammation in these diseases.

Peptides derived from different HSP27 variants may be effective in combating NDs. A study exploring the binding mechanisms of HSP27 to α-syn used two variants of HSP27: one that mimics the phosphorylated form of HSP27 and the other containing only the α-crystallin structure. Both peptide variants were identified as chaperone-active, as they can prevent the aggregation of monomeric α-syn. Among these two HSP27 variants, α-syn aggregation occurs, as evidenced by its co-sedimentation with the fibrils, indicating its binding affinity with the fibrils [190, 191]. Conversely, the peptide that contains only the α-crystallin structure is unable to interact stably with the fibrils due to the absence of the N- and C-terminal regions of HSP27[190].

7.4. Therapy Targeting CMA

A promising therapeutic strategy for PD involves enhancing CMA to clear pathogenic proteins like α-syn.

This can be achieved by upregulating the key CMA receptor LAMP2A, potentially using compounds like geldanamycin or metformin. Furthermore, modifying pathogenic proteins with additional KFERQ motifs can actively recruit them for CMA degradation, offering a targeted therapeutic approach to restore proteostasis and provide neuroprotection [192, 193].

Various small molecules or drugs have been identified that improve the survival of neurons in neurodegenerative conditions by modulating the activity of CMA. Metformin upregulates CMA by activating the TAK1-IKKα/β signaling axis, leading to phosphorylation of HSC70 at Serine 85. This enhances the interaction of CMA substrates with the lysosomal receptors HSC70 and LAMP2A, promoting their degradation[194]. However, findings on its long-term cognitive impact are conflicting. One study demonstrated contrasting results, reporting that extended metformin use worsened memory retention and visual discrimination in both aged normal mice and 3×Tg-AD model mice [195, 196]. The MAP trial (Metformin in Alzheimer's dementia Prevention), a phase II/III RCT, is ongoing, testing whether extended-release metformin (2000 mg/day) can prevent cognitive decline in 326 people with amnestic MCI but without diabetes. Caffeine, a natural compound prevalent in coffee, tea, and cocoa, has been demonstrated to mitigate pathological alterations induced by α-syn in models of PD. Mechanistically, chronic caffeine administration has been shown to restore CMA function by counteracting the α-syn-induced downregulation of LAMP2A [197]. Separately, silymarin—a BBB-permeable molecule with recognized antioxidant properties in the CNS—was found to upregulate both LAMP2 and LAMP2A expression in the MPTP mouse model of PD [198]. This enhanced autophagy pathway contributed to the prevention of mitochondrial dysfunction and oxidative stress. Hence, the overall α-syn level decreases in the mouse model, showing the therapeutic function of silymarin in enhancing the presence of LAMP2A and thus increasing CMA activity [199]. Silymarin exhibits strong neuroprotective potential in AD by reducing Aβ/tau pathology, oxidative stress, and neuroinflammation. Recent clinical trials have shown improved memory in individuals with mild cognitive impairment at a dose of 250 mg twice daily for 16 weeks [200].

7.5. Heat Therapy

Passive heating through body warming is a form of heat therapy that can upregulate HSPs, including HSP70 and HSP90. Research in healthy humans shows that 40 min of 39 °C local thigh heating raises HSP70 and HSP90α mRNA ~2.3-fold in vastus lateralis. Independently, 30 min of shear stress at 15 dyn cm^{-2} evoked a 1.8-fold increase. During cycling that drives core to 38.5–39.0 °C, intracellular HSP70 protein doubles and plasma HSP70 rises from 2 to 8 ng mL^{-1} within 60 min [201-203]. Studies on individuals engaging in body warming activities, such as sauna bathing and regular exercise, have shown a lower risk of developing NDs such as AD. Furthermore, PD and AD patients have demonstrated reduced symptoms and slowed disease progression through exercise-mediated passive heating activities such as walking and cycling [201].

7.6. Overcoming the Blood-Brain Barrier

A major challenge in treating NDs is effectively delivering therapeutics across the BBB. Although promising, many molecular agents, such as recombinant proteins and gene therapies, face obstacles due to the BBB's selective permeability. Therefore, creating innovative delivery platforms is essential for turning HSP-based strategies into successful CNS treatments.

7.6.1. Intranasal Administration of HSP

Intranasal administration is a non-invasive method that enables drugs to bypass the BBB and enter the CNS directly. This route leverages the unique anatomy of the nasal cavity, where the olfactory and trigeminal nerves provide a conduit from the nasal epithelium to the brain parenchyma and cerebrospinal fluid [204]. The respiratory region, with its high permeability and large surface area, further promotes efficient absorption.

Given these advantages, intranasal delivery of HSP70 has been investigated as a promising therapeutic strategy. Studies have confirmed that exogenous recombinant human HSP70 administered intranasally can successfully penetrate the murine brain, with detectable levels in critical regions such as the hippocampus, cerebellum, locus coeruleus, and olfactory bulbs [205]. This direct delivery method translates to functional benefits. For instance, Ribeiro et al. demonstrated that intranasal HSP70 protected against 6-OHDA-induced dopaminergic denervation in a PD model, reducing apomorphine-induced rotations and motor deficits [74]. These findings validate intranasal delivery as a viable route for administering neuroprotective chaperones.

7.6.2. Direct Protein Administration

While direct administration of recombinant proteins is a straightforward concept, its efficacy is limited by rapid degradation, poor cellular targeting, and inefficient traversal of biological barriers. To address these challenges, EVs—natural, cell-derived nanocarriers—have emerged as an advanced delivery platform. EVs can

encapsulate therapeutic proteins, protecting them from degradation and facilitating their efficient and selective uptake by target cells [206].

The potential of EV-mediated HSP delivery has been demonstrated in HD models. One study showed that small EVs (sEVs) successfully delivered the chaperone DNAJB6 via intrathecal administration in HD transgenic mice. This sEV-based delivery system protected DNAJB6 from degradation, facilitated its entry into brain tissue, and effectively suppressed polyQ peptide aggregation [207]. This approach highlights a powerful strategy to overcome the limitations of direct protein therapy, ensuring that therapeutic chaperones reach their site of action within the CNS.

7.7. Gene Therapy

Gene therapy is a promising therapeutic tool that can provide prolonged therapeutic effects. It involves correcting defective genes by transferring a copy of functional therapeutic genetic material to affected cells. The cells can maintain the gene through their normal cellular machinery of transcription and translation[208]. Some standard gene therapies that address genetic defects include suppressing mutant alleles via RNA interference (RNAi), adding functional genes, employing gene-editing techniques, and introducing disease-modifying genes[209].

7.7.1. CRISPR (clustered regularly interspaced short palindromic repeat)/Cas9 Editing

CRISPR/Cas9 is a promising gene-editing tool for NDs like AD, PD, HD, and ALS. It enables precise genome modifications by targeting specific genes. In AD, it corrects mutations in presenilin (PSEN1) and APP, reducing amyloid-beta and tau pathology [210]. For PD, it edits synuclein alpha (SNCA) and LRRK2 to alleviate α-syn aggregation and neurodegeneration [211, 212]. In HD, it suppresses mHtt expression, while in ALS, it targets SOD1 and C9orf72 to correct genetic defects [211]. Delivery methods include AAV and non-viral approaches, such as mRNA or protein-based systems. EVs have the potential to enhance delivery efficiency [213]. Despite challenges like off-target effects and safety concerns, CRISPR/Cas9 offers a transformative approach to treating NDs.

7.7.2. Gene Vector

The possible viral vectors tested include herpes simplex virus (HSV), vaccinia, polio, measles, vesicular stomatitis virus (VSV), and AAV. Among all viral vectors, AAV is the preferred delivery vehicle for in vivo gene delivery due to its advantages [209].

AAVs are part of the Parvoviridae family and are characterized by their lack of an envelope and a single-stranded linear DNA genome. The therapeutic use of AAV vectors in gene therapy for blood cancers and genetic forms of blindness has been approved by the FDA. These recombinant viruses are nonpathogenic and do not elicit significant immune responses in humans. It has been shown that transgene expression in the CNS of a human can last up to 4 years [214]. While using biomolecules including nucleic acids, peptides and proteins for treatment faces challenges such as poor cellular uptake, instability and pharmacokinetic properties, AAV-mediated gene therapy offers solutions to these limitations [215].

As a protein-based nanoparticle, recombinant AAV (rAAV) can effectively deliver DNA cargo to the nucleus of the cells by traveling across the cell membrane. This process begins when AAV at the cell surface is recognized by glycosylated receptors, triggering clathrin-mediated endocytosis into endosomes. As pH of the endosome decreases, conformational changes in the VP1/VP2 regions result in the escape of AAV into the nucleus. Once there, the uncoated AAV exists in two forms: single-stranded AAV (ssAAV), which requires conversion to double-stranded DNA before transcription, and self-complementary AAV (scAAV). Circular episomal genomes then form via intra- and inter-molecular recombination and integrate into the host genome [153].

AAV vectors are a prominent tool for delivering therapeutic genes, including those encoding HSPs, in NDs. A key advancement in their application is convection-enhanced delivery (CED), an infusion technique often guided by magnetic resonance imaging. CED utilizes a pressure gradient to generate bulk flow within the brain parenchyma, enabling safe administration with widespread vector distribution and minimal tissue damage due to its low infusion velocity [209].

Moloney et al. demonstrated that AAV-mediated gene transfer robustly increases HSP expression [216]. In a PD rat model, stereotaxic injection of AAV vectors carrying HSP70 or HSP27 led to successful transgene expression in nigral dopaminergic neurons [54]. The overexpression of HSP70 was particularly effective, reducing α-synuclein-induced axonal pathology in the striatum. Similarly, in an idiopathic PD mouse model, AAV-HSP70 gene transfer conferred significant protection to the dopaminergic system, preventing neuron loss, maintaining striatal dopamine levels, and preserving tyrosine hydroxylase-positive fibers [217]. Beyond HSPs, AAV vectors can also be used to deliver other therapeutic agents, such as neurotrophic factors, or to model PD by

overexpressing pathogenic proteins like α-syn in specific brain regions like the striatum or substantia nigra [218].

7.7.3. RNAi-Based Gene Therapy

RNAi mediates innate immunity and gene regulation via small non-coding RNAs, primarily microRNAs (miRNAs) and small interfering RNAs (siRNAs) [219]. miRNAs facilitate intercellular communication and post-transcriptional silencing [220] through a defined biogenesis cascade: RNA polymerase II synthesizes pri-miRNA, Drosha cleaves pre-miRNA, Exportin-5 exports pre-miRNA, Dicer processes mature miRNA, and mature miRNA loads into the RNA-induced silencing complex (RISC) complex (Argonaute proteins) to target mRNAs [221].

Several miRNAs are involved in this process by targeting HSPs: miR-1 targets HSP70 and HSP60 during oxidative stress to promote apoptosis, while miR-378 regulates HSF1, which is essential for HSP expression. Additionally, in neuronal cells, miR-3120 interacts with various conserved sites within the 3' UTR of HSC70/HSPA8, modulating HSC70 levels [222]. Due to their critical regulatory functions, miRNAs represent promising therapeutic targets for neurodegenerative diseases [223]. AMT-130, an engineered miRNA therapy, reduced mHtt in HD minipig models and is currently undergoing Phase I/II clinical trials in early-stage HD patients [209].

Although no studies have yet focused on RNAi-based gene therapy to enhance HSP expression by silencing genes, this approach shows potential to regulate HSP levels by suppressing genes that inhibit the HSR pathway, CMA, or HSP expression.

8. Clinical Trials on HSP Pathway in NDs

8.1. Arimoclomol

Arimoclomol is a drug that enhances the induction of HSPs by acting as an HSP co-inducer. It shows great potential in managing various NDs. Clinical trials on Arimoclomol in patients with conditions such as ALS and NPC have been conducted to evaluate its effectiveness and safety.

In ALS, results were contingent on the trial phase. A phase II trial demonstrated a favorable safety profile and suggested therapeutic potential at various concentrations, highlighting the drug's ability to cross the BBB [224]. However, a subsequent phase III trial in early ALS patients administering 1200 mg/day of Arimoclomol citrate for 76 weeks failed to show a significant therapeutic benefit, with higher doses proving poorly tolerated [225]. In contrast, Arimoclomol showed promising results in NPC, a rare neurodegenerative disorder. A phase II/III trial where patients received oral Arimoclomol three times daily for 12 months demonstrated a statistically significant 65% reduction in annual disease progression. The treatment was well-tolerated and its efficacy is attributed to the drug's ability to enhance lysosomal activity, amplify the HSR, and reduce lipid accumulation [226].

8.2. HSP60-Derived Peptides

HSP60-derived peptides are known to have immunomodulatory effects by regulating T-cell activity. These peptides have been widely researched as possible treatment interventions for type 1 diabetes. They can also be a potential treatment for NDs such as AD and PD.

The HSP60-derived peptide DiaPep277 has demonstrated a strong safety profile and functional efficacy in clinical trials for type 1 diabetes, an immune-mediated condition [227, 228]. This success poses a critical question: could similar HSP60-targeting strategies be effective in NDs with immune components? Currently, clinical data in this area are lacking, underscoring a significant opportunity for focused research and trials to translate this immunological insight into novel neuroprotective therapies.

8.3. HSP70 Modulators

Methylene blue, an HSP70 modulator, has advanced to Phase III clinical trials for AD following promising Phase II results. In a Phase II trial involving 321 AD participants, six months of treatment with methylene blue improved cognitive performance compared with placebo. After one year of treatment, the drug was associated with a remarkable 81% slowing of disease progression [229].

The therapeutic mechanism involves the specific oxidation of Cys306 in HSP70, which inhibits its ATPase activity. This locks HSP70 in an ADP-bound, high-affinity conformation for misfolded proteins, such as tau, leading to their subsequent degradation via the ubiquitin-proteasome pathway [230]. Given its demonstrated potential to interfere with tau aggregation, methylene blue represents a promising candidate not only for AD but also for other tauopathies, warranting further investigation in these conditions [231].

9. Conclusion

HSPs represent a highly promising therapeutic avenue for NDs by directly countering the fundamental pathology of protein aggregation and its associated cytotoxicity. Their diverse functions—including dissociating aggregates, facilitating the degradation of misfolded proteins, and

assisting in refolding—collectively maintain proteostasis and promote neuronal survival through a concerted network of distinct HSP families.

Despite the theoretical promise of HSPs as therapeutic targets for NDs, their clinical translation faces three fundamental obstacles that are underappreciated in the current literature. First, HSP90 inhibitors, while capable of activating HSP70-mediated protein degradation, exhibit severe off-target toxicity due to their broad client protein spectrum (>200 kinases and transcription factors), causing hepatotoxicity, cardiac QT prolongation, and oncogenic risks that have terminated multiple oncology trials [117, 118]. Second, the HSR harbors intricate compensatory feedback loops: chronic HSP70 overexpression transcriptionally represses HSF1 activity, while sustained HSF1 agonists, such as Arimoclomol, can trigger "chaperone exhaustion" through epigenetic silencing and proteasomal degradation of HSF1, ultimately accelerating proteostatic collapse [9]. This molecular tolerance likely underpins the devastating failure of Arimoclomol in ALS Phase III trials, where peripheral HSP70 induction failed to rescue motor neurons that had already lost HSF1 responsiveness, revealing a critical mismatch between biomarker signals and neuronal viability [149, 232]. Third, the BBB prevents peripheral HSP levels from reflecting central pathology, while late-stage neurons possess an irreversibly depleted chaperone network, rendering HSR reactivation futile [233, 234].

Organizing HSP-based interventions by disease stage reveals not a therapeutic roadmap, but a sequence of escalating biological constraints that systematically limit translational feasibility. This chronological framework allows us to view the diminishing returns of HSP modulation as neurodegeneration progresses.

1. Pre-Symptomatic Stage

Therapeutic rationale at this stage focuses on "fortifying" declining HSR capacity through lifestyle interventions (heat therapy, exercise) and dietary compounds (resveratrol) that non-specifically elevate peripheral HSP70/90. Chemical chaperones like TUDCA are positioned for high-risk genotypes (e.g., HSPB1 mutations). However, the translational value here remains purely hypothetical. The age-related decline in HSF1 activity is not a reversible deficit, and we lack biomarkers to verify that peripheral induction translates to neuronal HSR restoration. Without CSF or imaging-based assays of HSF1 functional competency, these strategies amount to empirical guesswork, delivering pharmacodynamic signals of uncertain CNS impact.

2. Early Symptomatic Stage

Once proteostatic failure manifests clinically, the logical shift is to active modulation: rimoclomol amplifies residual HSF1 activity, HSP90 inhibitors forcibly derepress the HSR, and silymarin/GGA enhance CMA-mediated aggregate clearance. This stage, however, exposes the critical translational flaw: forced HSR amplification triggers self-attenuating feedback loops. Chronic HSP70 overexpression transcriptionally silences HSF1, while sustained agonism induces chaperone exhaustion via proteasomal degradation and epigenetic repression. The catastrophic failure of rimoclomol in ALS Phase III trials—where robust peripheral HSP70 induction failed to rescue motor neurons with lost HSF1 responsiveness—demonstrates that biomarker decoupling is inevitable. Amplifying a failing system accelerates its collapse, not its recovery.

3. Established Disease

In late-stage disease, direct protein delivery (intranasal HSP70), gene therapy (AAV-HSP vectors), and EV-mediated chaperone transport represent technological solutions to a misdefined problem. Neurons burdened by widespread aggregation and mitochondrial collapse do not suffer from inadequate chaperone supply; they suffer from irreversible HSR infrastructure failure. The translational value of these advanced platforms is negligible because bypassing the BBB cannot restore transcriptional unresponsiveness or proteasomal insufficiency. Treating low HSP levels while ignoring the dead regulatory circuitry is a strategy of diminishing biological logic.

Viewed chronologically, the translational promise of HSPs contracts not from a shortage of candidates, but because the therapeutic target—the inducible neuronal HSR—dissolves stage-by-stage. This perspective reframes the central challenge: future research must abandon global HSP induction and instead develop precision stratification tools that assess neuronal HSF1 functional capacity, coupled with modalities (e.g., molecular glues, RNAi) that bypass rather than futilely hyperactivate a defunct proteostatic network.

Author Contributions

EYY, DLH, TCG and ZDZ reviewed the literature and drafted the manuscript. XXD, LZ, SL, YCW, and EKT provided critical comments, revised, and touched up the manuscript. All authors have read and agreed to the submitted version of the manuscript.

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Conflicts of Interest

The authors declare that they have no competing interests.

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