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REVIEW

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Proteostasis disruption under hypoxia: therapeutic targets in cancer and neurodegenerative diseases

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ABSTRACT

Proteostasis, the integrated network regulating protein synthesis, folding, trafficking, and degradation, is essential for cellular function and organismal health. Reduced oxygen availability disrupts proteostasis through increased reactive oxygen species (ROS) production, endoplasmic reticulum (ER) stress, impaired ATP-dependent protein folding, and altered chaperone expression. In cancer, tumor cells exploit chronic unfolded protein response (UPR) signaling to enhance survival, angiogenesis, and therapeutic resistance. Inhibition of IRE1α and PERK pathways has shown efficacy in preclinical models, though clinical translation faces challenges including off-target toxicity. In neurodegenerative diseases—Alzheimer's, Parkinson's, and amyotrophic lateral sclerosis—chronic hypoxia accelerates protein aggregate accumulation through oxidative modifications and impaired autophagy-lysosome function. Therapeutic strategies targeting γ-secretase, BACE1, and protein clearance pathways have demonstrated limited clinical success despite mechanistic rationale. Understanding hypoxia-induced proteostasis failure may inform therapeutic development, though significant obstacles remain in translating preclinical findings to effective treatments for cancer and neurodegenerative diseases.

Keywords: protein homeostasis, oxygen deprivation, unfolded protein response, protein aggregation, hypoxia-inducible factor

Introduction

Eukaryotic organisms express thousands of distinct proteins that require sophisticated regulatory mechanisms to maintain cellular function. Proteostasis—protein homeostasis—encompasses the biological pathways regulating protein synthesis, folding, trafficking, and degradation to maintain proteome balance [1,2]. This network is essential for organismal development and cellular function across tissues.

The proteostasis network ensures proteins are correctly folded, delivered to appropriate cellular locations, and that misfolded or excess proteins are efficiently removed through autophagy or ubiquitin-proteasome-mediated degradation [3]. When these quality control mechanisms fail, protein aggregates accumulate, contributing to cancer, neurodegenerative diseases, and aging-related disorders [4].

Maintaining proteome balance becomes difficult under environmental and physiological stressors. Hypoxia—reduced oxygen availability—represents one such condition. Hypoxia impairs cellular function by decreasing aerobic respiration rates and compromising adenosine triphosphate (ATP) production. Beyond energy depletion, hypoxia threatens protein integrity through oxidative stress and impaired disulfide bond formation, disrupting proteostatic mechanisms [5].

This review addresses three questions: (i) How does hypoxia disrupt cellular proteostasis networks? (ii) What are the consequences of hypoxia-induced proteostasis failure in cancer and neurodegenerative diseases? (iii) How can understanding these mechanisms inform therapeutic strategies? By synthesizing current evidence on proteostasis under hypoxic conditions, we identify potential therapeutic targets for diseases characterized by

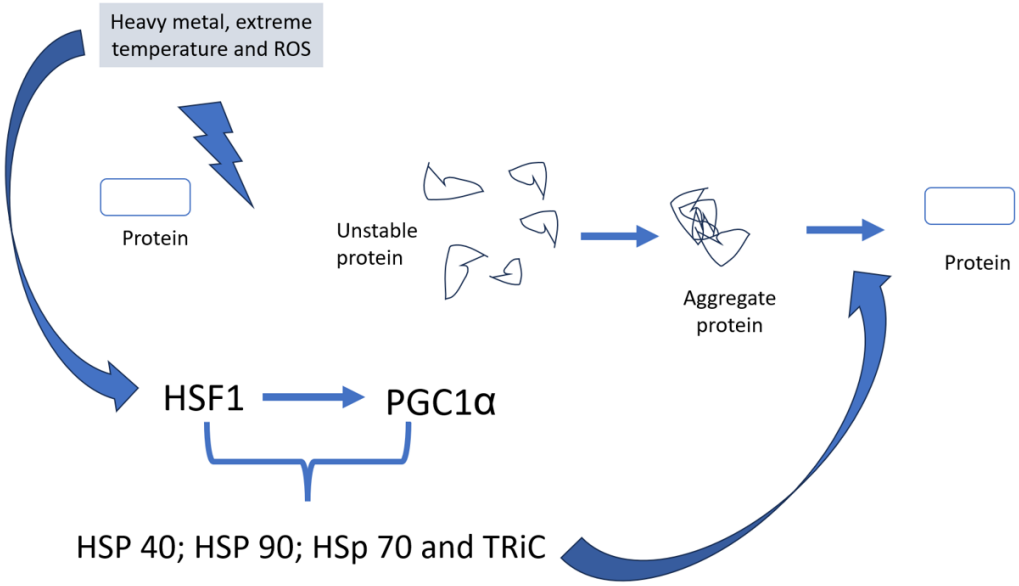

Figure 1. Mechanisms maintaining protein conformational stability. The proteostasis network responds to thermal and metabolic stress through coordinated activation of transcription factors (HSF1, PGC1α) and metabolic sensors (AMPK). HSF1 activation leads to upregulation of heat shock proteins including HSP40, HSP70, HSP90, and TRiC, while PGC1α cooperates with HSF1 to enhance chaperone expression. This coordinated response prevents protein aggregation and promotes refolding of stress-damaged proteins, maintaining proteome balance under adverse conditions.

proteome imbalance, though significant challenges remain in translating preclinical findings to clinical applications.

Proteostasis: cellular protein homeostasis

Overview of proteostasis

Proteins represent one of the most abundant and functionally diverse biomacromolecules in cells, serving roles in enzymatic catalysis, structural support, cellular motility, immune defense, and signal transduction [6]. Human cells synthesize an estimated 10,000 distinct protein types [7], making protein balance essential for metabolic stability and cellular function.

Proteostasis encompasses several interconnected processes: protein synthesis and folding, conformational stability maintenance, trafficking to appropriate cellular compartments, and degradation of damaged or unnecessary proteins. When functioning properly, the proteostasis network prevents protein misfolding and efficiently removes aberrant proteins, thereby preventing formation of toxic protein aggregates that can disrupt cellular function and contribute to disease pathogenesis [1].

Protein synthesis and folding

Most proteins must fold into specific three-dimensional conformations to achieve biological activity. This native structure represents the thermodynamically favorable state for each protein. However, protein folding does not occur spontaneously in the crowded cellular environment. Instead, it requires assistance from molecular chaperones that facilitate efficient folding while preventing aggregation [3].

Molecular chaperones interact with nascent polypeptide chains emerging from ribosomes, recognizing exposed hydrophobic amino acid residues that would otherwise promote aggregation. Through cycles of ATP-dependent or ATP-independent binding and release, chaperones guide proteins through the folding landscape toward their native conformations [1]. Additionally, chaperones prevent premature folding before polypeptides reach their target organelles and protect cytosolic domains of transmembrane proteins destined for the endoplasmic reticulum (ER) and other organelles from aggregation during transit [3].

Defense of conformational stability

Folded protein structures exist in metastable states, vulnerable to destabilization by genetic mutations, heavy metals, elevated temperatures, and reactive oxygen species (ROS). Under these stress conditions, proteins may partially or completely unfold, exposing hydrophobic residues and unpaired β-strands that promote aggregation through inappropriate intermolecular interactions [2].

The cellular proteostasis network can be rapidly mobilized in response to environmental stresses such as heat shock or metabolic perturbation. During stress conditions, transcription factors called heat shock factors (HSFs) are released from inhibitory complexes and activate genes encoding heat shock proteins and other proteostasis network components [8].

Heat shock transcription factor 1 (HSF1) directly induces expression of peroxisome proliferator-activated receptor gamma coactivator 1α (PGC1α). Both HSF1 and PGC1α are coordinately regulated by the metabolic stress sensor 5′-AMP-activated protein kinase (AMPK). AMPK phosphorylates HSF1, modulating its activity and initiating a proteotoxic stress response. PGC1α cooperates with HSF1 to activate expression of multiple chaperones including HSP40, HSP70, HSP90, and TRiC, thereby enhancing the cell's capacity to refold stress-damaged proteins [8]. Several heat shock protein families are constitutively expressed in the cytosol and organelles to maintain surveillance of the proteome and mediate refolding of non-native proteins—those that have not achieved their functional folded structure or proper cellular localization [1].

Protein degradation pathways

Protein degradation is essential to prevent accumulation of misfolded proteins that could form toxic aggregates. Cells employ two major degradation pathways: the ubiquitin-proteasome system (UPS) and the autophagosomal-lysosomal pathway [9].

Ubiquitin-proteasome system (UPS)

The UPS represents the primary pathway for selective degradation of short-lived and misfolded proteins. This ATP-dependent process requires proteins to be tagged with polyubiquitin chains, which serve as recognition signals for the 26S proteasome complex. The proteasome, a large multi-subunit protease, processively degrades ubiquitinated proteins into small peptides [9].

Substrates for UPS-mediated degradation include: (1) Misfolded cytosolic proteins exposed to heat stress, oxidative stress, or heavy metal toxicity; (2) Truncated or aberrant proteins produced from defective messenger ribonucleic acids (mRNAs) lacking stop codons, which are identified through ribosome quality control mechanisms and targeted for proteasomal degradation, and (3) Secretory proteins that fail to fold properly in the oxidizing environment of the ER and are retrotranslocated to the cytosol for ER-associated degradation (ERAD) [10,11].

Autophagosomal-lysosomal pathway

Autophagy represents a complementary degradation pathway that does not require ATP for substrate recognition and can process both intracellular proteins (macroautophagy) and extracellular material internalized by endocytosis (heterophagy). This pathway is important for clearing large protein aggregates, damaged organelles, and long-lived proteins that cannot be efficiently processed by the proteasome [9]. The balance between UPS and autophagy determines cellular capacity to maintain proteostasis, with autophagy becoming increasingly important when the UPS is overwhelmed or when large aggregates form that cannot enter the proteasome barrel structure [12].

Impact of proteostasis disruption

Disruption of the proteostasis network influences pathologies associated with aging, neurodegenerative diseases, and cancer. Protein aggregates that escape quality control mechanisms can interact with numerous cellular proteins, including

ribonucleoproteins, nucleocytoplasmic transport factors, and essential metabolic enzymes [13]. These interactions sequester functional proteins and disrupt cellular processes.

Proteins prone to aggregation typically contain metastable domains, intrinsically disordered regions, or low-complexity sequences that increase their tendency to misfold and aggregate. Importantly, protein aggregates themselves can recruit and sequester molecular chaperones and other proteostasis components, creating a cycle that further compromises the cell's ability to mount an effective stress response [12]. This depletion of available chaperones impairs the heat shock response, which normally serves as a defense mechanism for maintaining proteome balance [4].

Under normal conditions, the proteostasis network employs multiple strategies to manage misfolded proteins: (i) Chaperones bind to exposed hydrophobic surfaces of misfolded proteins, preventing aggregation and facilitating refolding or degradation. (ii) Pre-existing small oligomers are protected by chaperones like small heat shock proteins (sHSPs) to prevent interactions with other cellular proteins. (iii) Toxic soluble oligomers are converted into larger, less reactive inclusion bodies composed of fibrillar or amorphous aggregates, reducing the surface area available for aberrant protein-protein interactions. (iv) Existing aggregates are disaggregated by specialized chaperones, and the resulting proteins are either refolded or targeted for degradation via UPS or autophagy. (v) Aggregation-prone proteins may be sequestered in specific cellular compartments (nucleus, mitochondria) that are more resistant to aggregation-induced toxicity than the cytosol [2].

In dividing cells, asymmetric partitioning during mitosis can segregate protein aggregates to one daughter cell, creating a rejuvenated daughter cell with reduced aggregate burden. However, this protective mechanism is unavailable to postmitotic cells such as neurons, rendering them vulnerable to aggregate-related pathologies [2].

During aging, the capacity to maintain proteome balance progressively declines, contributing to age-related degenerative diseases. This decline is characterized by accumulation of protein aggregates, loss of protein solubility, and decreased expression or activity of key proteostasis components [2]. The proteostasis network becomes increasingly burdened by rising numbers of misfolded proteins and proteins damaged by cumulative oxidative stress, highlighting the importance of proteostasis in health and disease [14].

Hypoxia and cellular responses

Definition and molecular mechanisms

Hypoxia is defined as a state of reduced oxygen availability in cells or tissues, insufficient to meet metabolic demands. In mammals, oxygen homeostasis and adaptation to hypoxic conditions are primarily mediated by hypoxia-inducible transcription factors (HIFs), particularly the HIF-1 complex [5].

Under normoxic conditions (physiological oxygen levels), the HIF-1α subunit is continuously synthesized but rapidly degraded. This degradation is mediated by oxygen-dependent prolyl hydroxylase domain-containing proteins (PHDs), which hydroxylate specific proline residues on HIF-1α. Hydroxylated HIF-1α is recognized by the von Hippel-Lindau (VHL) E3 ubiquitin ligase complex, leading to polyubiquitination and subsequent proteasomal degradation via the 26S proteasome pathway[5].

During hypoxia, PHD activity is inhibited due to insufficient oxygen, which serves as a co-substrate for the hydroxylation reaction. Stabilized HIF-1α accumulates, translocates to the nucleus, and heterodimerizes with HIF-1β (also known as aryl hydrocarbon receptor nuclear translocator or ARNT). This HIF-1α/HIF-1β complex binds to hypoxia-responsive elements (HREs) in the promoter regions of target genes, activating transcription of genes involved in angiogenesis such as vascular endothelial growth factor (VEGF), erythropoiesis (EPO), glucose metabolism (glucose transporter type 1 or GLUT1, glycolytic enzymes), and cell survival pathways [5].

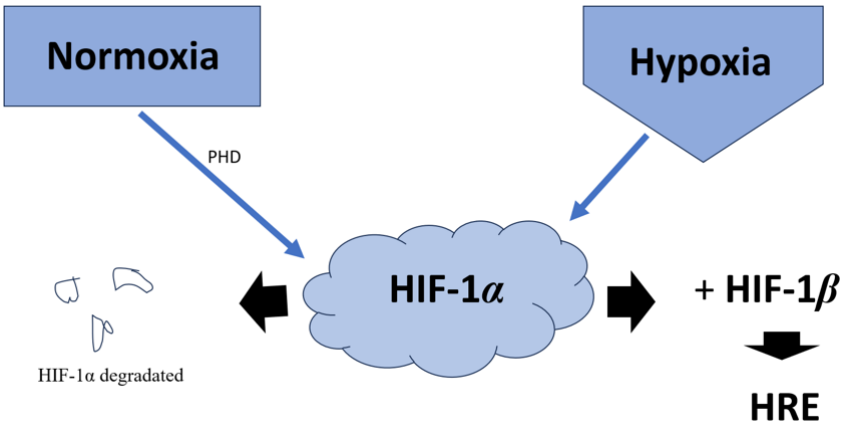

Figure 2. Regulation of HIF-1α under normoxic and hypoxic conditions. Under normoxia, oxygen-dependent prolyl hydroxylase (PHD) hydroxylates HIF-1α at specific proline residues, creating a recognition site for von Hippel-Lindau (VHL) E3 ubiquitin ligase. VHL ubiquitinates HIF-1α, targeting it for proteasomal degradation. Under hypoxia, reduced oxygen availability inhibits PHD activity, preventing HIF-1α hydroxylation. Stabilized HIF-1α accumulates, translocates to the nucleus, dimerizes with HIF-1β, binds to hypoxia-responsive elements (HREs), and activates transcription of genes involved in angiogenesis, glycolysis, and cellular adaptation to low oxygen conditions.

Hypoxia-induced oxidative stress

Paradoxically, hypoxia can increase reactive oxygen species (ROS) production despite reduced oxygen availability. This occurs primarily through mitochondrial electron transport chain dysfunction, where impaired complex III and IV activity leads to increased electron leakage and superoxide formation [5,15].

Elevated ROS levels under hypoxia threaten protein integrity through several mechanisms. Hypoxia interferes with cellular energy production by inhibiting oxidative phosphorylation, reducing ATP synthesis. This energy deficit compromises ATP-dependent proteostasis processes including chaperone-mediated folding and UPS-dependent degradation [15]. Simultaneously, ROS directly damage proteins through oxidation of amino acid side chains, particularly cysteine and methionine residues, formation of protein carbonyls, and generation of protein cross-links [15,16].

Heat shock protein response to hypoxia

To counteract proteotoxic stress under hypoxic conditions, cells upregulate expression and activity of heat shock proteins (HSPs). HSPs are molecular chaperones that play important roles in maintaining proteostasis by preventing aggregation, facilitating refolding, and directing irreversibly damaged proteins to degradation pathways [17].

HSPs can be functionally classified into three categories based on their mechanism of action: (i) Holdases recognize and stabilize partially collapsed proteins, prevent aggregation, and present proteins to foldases (e.g., small HSPs like HSP27, αB-crystallin). (ii) Foldases directly facilitate protein folding through ATP-dependent conformational cycles (e.g., HSP70, HSP90, HSP60, TRiC/CCT complex). (iii) Disaggregases disassemble protein aggregates and transfer partially folded proteins to holdases or foldases for reprocessing (e.g., HSP110-HSP70-HSP40 system in mammals) [18]. Alternatively, HSPs are commonly categorized by molecular weight (HSP27, HSP40, HSP60, HSP70, HSP90, HSP100), which roughly corresponds to their structural features and substrate specificity (Table 1).

Proteostasis under hypoxic conditions

Overview of hypoxia-induced proteostasis disruption

Hypoxic conditions disrupt cellular proteostatic networks through coordinated effects on protein synthesis, quality control, and degradation pathways. While cells attempt to maintain proteome functionality through adaptive responses, prolonged

Table 1. Major chaperone families and their functions in proteostasis

Chaperone family	Function	Classification
Heat shock protein 70 (HSP70)	Prevents aggregation; folds newly synthesized proteins; maintains native conformations; cooperates with HSP40 and HSP110 in disaggregation	Foldase (ATP-dependent)
Heat shock protein 40 (HSP40)	Recruits HSP70 to substrate proteins requiring folding	Co-chaperone
Heat shock protein 110 (HSP110)	Cooperates with HSP70 in folding and repairing misfolded proteins	Foldase/Disaggregase
Heat shock protein 90 (HSP90)	Functions as homodimer in folding functionally and structurally diverse proteins; involved in multiple cellular pathways	Foldase (ATP-dependent)
Heat shock protein 60 (HSP60)	Mitochondrial chaperone; folds imported cytosolic proteins within mitochondria	Foldase (ATP-dependent)
TCP-1 ring complex (TRiC)	Cytosolic chaperonin complex; folds proteins including actin and tubulin	Foldase (ATP-dependent)
Heat shock protein 100 (HSP100)	Disaggregase chaperones in bacteria, fungi, and chloroplasts	Disaggregase
Small heat shock proteins (sHSPs)	ATP-independent holdases; prevent aggregation by binding partially unfolded proteins; stabilize proteins for processing by foldases	Holdase (ATP-independent)

or severe hypoxia ultimately overwhelms protective mechanisms, leading to accumulation of misfolded proteins and toxic aggregates.

Multiple studies demonstrate that hypoxia affects proteostasis through several interconnected mechanisms: (i) global inhibition of protein translation to conserve energy and reduce proteotoxic load; (ii) selective upregulation of stress-responsive proteins including HSPs and UPR components; (iii) impaired protein folding due to ATP depletion and disrupted disulfide bond formation; and (iv) altered balance between protein degradation pathways [19–21].

Translational repression and selective protein synthesis

During hypoxia, cells rapidly suppress global protein translation as an adaptive response to conserve ATP and reduce the burden on protein folding machinery. This translational repression occurs primarily through phosphorylation of eukaryotic translation initiation factor 2α (eIF2α) by the ER stress kinase PERK (PKR-like ER kinase), which is activated as part of the unfolded protein response [20].

Phosphorylated eIF2α inhibits the guanine nucleotide exchange factor eIF2B, reducing the availability of eukaryotic initiation factor 2-guanosine triphosphate-initiator methionyl transfer RNA (eIF2-GTP-Met-tRNA) ternary complexes required for translation initiation. This results in global suppression of cap-dependent translation, reducing the flux of newly synthesized proteins entering the folding machinery. However, certain mRNAs with internal ribosome entry sites (IRES) or upstream open reading frames (uORFs) can bypass this inhibition, allowing selective translation of stress-responsive proteins including activating transcription factor 4 (ATF4), C/EBP homologous protein (CHOP), and various HSPs[20].

Studies in diverse organisms including Drosophila, turtles, and mammalian cell cultures have confirmed that protein synthesis rates decrease substantially during hypoxia [19]. Simultaneously, chaperone expression increases, creating a favorable ratio of chaperone capacity to newly synthesized proteins that helps maintain folding quality despite reduced total protein synthesis.

Differential regulation of heat shock proteins

A study by Wen et al. (2021) investigating Tibetan sheep adapted to high-altitude hypoxia revealed differential regulation of ATP-dependent

and ATP-independent chaperones under oxygen limitation [22]. This research provides insights into how organisms balance energy conservation with proteostasis maintenance during chronic hypoxia.

HSP27 (ATP-independent holdase)

HSP27 expression and protein levels increased substantially across all tissues examined (heart, liver, lung, kidney, skeletal muscle) in Tibetan sheep under hypoxic conditions compared to normoxia. This upregulation was maintained even during extreme hypoxia, when other energy-dependent processes were suppressed [22].

The sustained elevation of HSP27 reflects its role as an ATP-independent holdase chaperone. HSP27 forms large oligomeric complexes that bind to hydrophobic surfaces of partially unfolded proteins, preventing their aggregation without requiring ATP hydrolysis. By stabilizing misfolded proteins in a folding-competent state, HSP27 maintains these proteins in a reservoir that can be processed by ATP-dependent foldases when energy becomes available [18]. This energy-efficient strategy allows cells to maintain minimal proteostasis capacity during prolonged hypoxia without depleting scarce ATP reserves [22].

HSP60 (ATP-dependent foldase)

In contrast to HSP27, HSP60 showed a biphasic response to hypoxia. Under moderate hypoxia, HSP60 mRNA and protein levels increased, consistent with an adaptive stress response. However, under extreme hypoxic conditions, HSP60 expression decreased substantially across all examined tissues [22].

This downregulation reflects the high ATP cost of HSP60-mediated protein folding. HSP60, a member of the chaperonin family, operates through ATP-dependent cycles of substrate binding, encapsulation within its central cavity, and ATP-hydrolysis-driven conformational changes that facilitate protein folding [17,23]. Under extreme hypoxia, when ATP is severely limited and cells must prioritize energy allocation to essential processes such as ion homeostasis and nucleotide synthesis, the energetic cost of maintaining HSP60 activity becomes prohibitive [23].

By reducing HSP60 expression, cells conserve ATP for more essential survival functions, though this strategy compromises their capacity for active protein refolding [22,23]. The consequence is accumulation of proteins stabilized by HSP27 but unable to achieve their native folded state, contributing to progressive proteostasis failure during prolonged severe hypoxia.

Oxidative damage and protein modification

Hypobaric hypoxia (the type of hypoxia experienced at high altitude) induces substantial changes in cellular redox homeostasis, leading to protein oxidation and loss of muscle mass. Research by Chaudhary et al. (2012) and Agrawal and Rathor (2017) demonstrated that acute hypobaric hypoxia exposure causes skeletal muscle wasting through multiple proteostasis-disrupting mechanisms [16,24].

Thiol oxidation and redox imbalance

Under hypobaric hypoxia, cellular thiol content decreases as cysteine and methionine residues are oxidized while attempting to buffer increased ROS production [16]. These sulfur-containing amino acids serve as the first line of antioxidant defense, becoming sacrificially oxidized to protect other cellular components. Cysteine residues form disulfide bonds or sulfenic acid derivatives, while methionine is oxidized to methionine sulfoxide.

The depletion of reduced thiols disrupts redox homeostasis, allowing ROS to attack other susceptible amino acids. Aromatic amino acids including tyrosine and tryptophan are vulnerable: tyrosine becomes oxidized to dityrosine (forming abnormal cross-links between proteins), while tryptophan undergoes various oxidative modifications [16].

Protein carbonylation

A hallmark of oxidative protein damage is increased carbonyl content, resulting from oxidation

of amino acid side chains (particularly arginine, lysine, proline, and threonine) or through Michael addition of lipid peroxidation products [16]. Carbonylated proteins cannot be repaired and must be degraded, but high levels of carbonylation can overwhelm degradation capacity, leading to accumulation of oxidatively damaged proteins.

The accumulation of oxidized and carbonylated proteins triggers compensatory upregulation of chaperones including HSP70, HSP60, and glucose-regulated protein 78/binding immunoglobulin protein (GRP78/BiP), which serve as biomarkers of increased misfolded protein burden [16]. However, if oxidative damage exceeds the capacity of chaperones and degradation systems, protein aggregates form and contribute to cellular dysfunction.

Endoplasmic reticulum stress and unfolded protein response

Hypoxia-induced ROS production and oxidative protein damage affect the ER, the primary site for synthesis and folding of secretory and transmembrane proteins. The ER maintains an oxidizing environment necessary for disulfide bond formation, but this same environment makes ER proteins especially vulnerable to oxidative damage under hypoxic stress [20].

ER stress induction

Accumulation of misfolded proteins in the ER lumen exceeds the capacity of ER-resident chaperones (GRP78/BiP, GRP94, calnexin, calreticulin), triggering ER stress. This activates the unfolded protein response (UPR), a coordinated adaptive program involving three ER transmembrane sensors: PERK, inositol-requiring enzyme 1α (IRE1α), and activating transcription factor 6 (ATF6) [20,25]

Under non-stressed conditions, GRP78/BiP binds to the luminal domains of PERK, IRE1α, and ATF6, keeping them inactive. When misfolded proteins accumulate, GRP78/BiP is titrated away to assist in their folding, relieving inhibition of the three UPR sensors and initiating downstream signaling cascades.

UPR signaling branches

The UPR operates through three distinct but coordinated signaling pathways. The PERK pathway initiates rapid translational reprogramming: activated PERK phosphorylates eIF2α, causing global translational repression while allowing selective translation of ATF4 (activating transcription factor 4). ATF4 induces genes involved in amino acid metabolism, antioxidant responses, and apoptosis (including CHOP) [20].

In parallel, the IRE1α pathway enhances ER protein folding capacity through its endoribonuclease activity that splices X-box binding protein 1 (XBP1) mRNA, generating the active transcription factor XBP1s (spliced XBP1). XBP1s induces genes encoding ER chaperones, components of ER-associated degradation (ERAD), and lipid biosynthesis enzymes to expand ER capacity [25].

The third branch, the ATF6 pathway, operates through proteolytic activation: under ER stress, ATF6 translocates to the Golgi apparatus where it is cleaved by site-1 and site-2 proteases (S1P and S2P), releasing the ATF6 fragment (ATF6f) that translocates to the nucleus and activates genes encoding ER chaperones and ERAD components [25]. Together, these three pathways coordinate cellular responses to restore ER homeostasis.

From adaptation to cell death

Initially, UPR activation is adaptive, enhancing protein folding capacity, increasing degradation of misfolded proteins, and reducing new protein synthesis to restore ER homeostasis. However, if ER stress is prolonged or severe, as occurs during chronic hypoxia, the UPR shifts from pro-survival to pro-apoptotic signaling. Sustained PERK-ATF4 signaling induces CHOP, a transcription factor that promotes apoptosis by downregulating the anti-apoptotic protein Bcl-2 and increasing expression of pro-apoptotic proteins [26].

Skeletal muscle wasting under hypoxia

The mechanisms of hypoxia-induced muscle wasting illustrate how proteostasis disruption

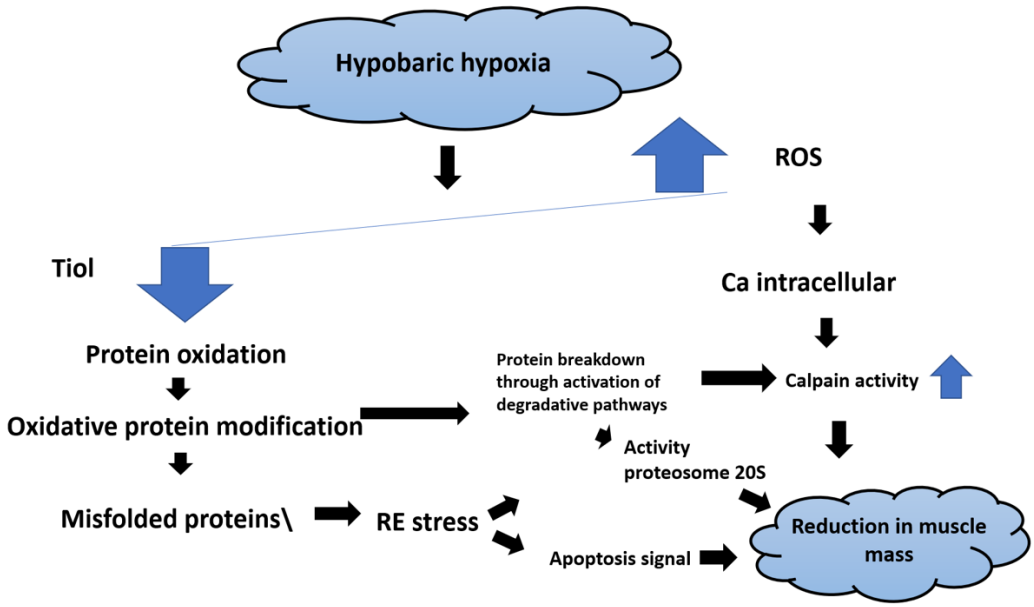

Figure 3. Mechanisms of hypoxia-induced muscle protein loss. Hypobaric hypoxia triggers multiple converging pathways leading to skeletal muscle wasting. (1) Increased ROS production causes thiol oxidation, depleting cysteine and methionine while promoting protein carbonylation and formation of dityrosine cross-links. (2) Oxidized proteins accumulate in the ER, triggering ER stress and activation of the unfolded protein response (UPR). (3) ER stress-induced calcium dysregulation activates calcium-dependent calpains, which cleave myofibrillar proteins. (4) Sustained ER stress activates apoptotic pathways through CHOP induction. (5) Accumulation of damaged proteins increases activity of both the 20S and 26S proteasomes. These converging mechanisms result in net muscle protein degradation exceeding synthesis, manifesting as muscle atrophy and functional impairment.

translates into tissue-level pathology. Multiple proteolytic pathways are activated simultaneously, overwhelming the muscle's capacity to maintain protein synthesis and leading to net protein loss.

Calcium dysregulation and calpain activation

Hypoxia-induced ROS production disrupts ER calcium homeostasis, causing calcium release into the cytoplasm [27]. Elevated cytosolic calcium activates calpains, calcium-dependent cysteine proteases that cleave myofibrillar proteins including titin, nebulin, and dystrophin, initiating muscle protein degradation [28]. Calpains also cleave and activate other proteolytic enzymes, amplifying the degradative response [28].

ER stress-induced apoptosis

Prolonged ER stress activates apoptotic pathways through several mechanisms, contributing to myonuclear apoptosis and loss of muscle fiber regenerative capacity [26].

Proteasomal degradation

The accumulation of misfolded proteins stimulates activity of the 20S proteasome (the catalytic core of the 26S proteasome), which can degrade oxidized proteins without ubiquitin tagging [16]. This ubiquitin-independent pathway, combined with traditional ubiquitin-dependent proteasomal degradation, contributes to net protein loss in muscle tissue.

Therapeutic approaches targeting proteostasis

Cancer: exploiting proteostasis addiction

Hypoxia is a common feature of solid tumors, arising when rapidly proliferating cancer cells outgrow their blood supply. Tumor hypoxia is associated with poor prognosis, resistance to radiation therapy and chemotherapy, increased metastatic potential, and selection for aggressive cancer cell phenotypes [29]. Understanding how cancer cells adapt to hypoxic stress through

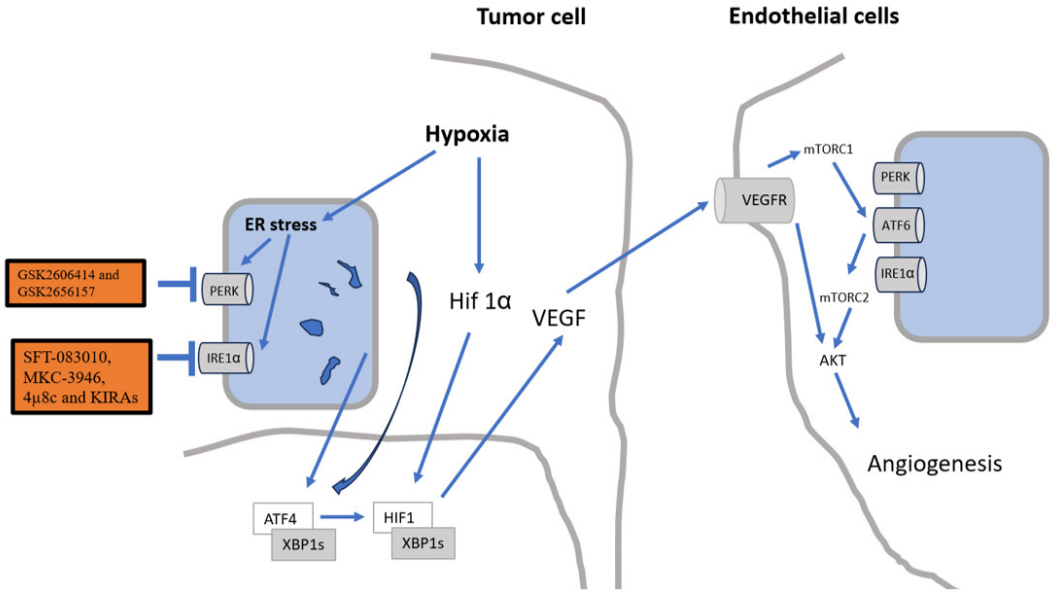

Figure 4. Strategies for inhibiting upr-mediated angiogenesis in cancer. Tumor hypoxia activates ER stress in cancer cells, leading to activation of UPR sensors PERK, IRE1α, and ATF6. PERK and IRE1α induce expression of transcription factors ATF4 and XBP1s, which upregulate VEGF expression. Secreted VEGF acts on endothelial cells, activating VEGFR and downstream PI3K-AKT signaling. VEGF also activates mTORC1, which phosphorylates ATF6 and PERK. These activated UPR sensors enhance AKT phosphorylation through mTORC2 activation, creating a positive feedback loop promoting angiogenesis. Therapeutic interventions target multiple nodes: (1) Direct IRE1α RNase inhibitors (STF-083010, MKC-3946, 4μ8c) block XBP1 splicing; (2) Kinase-inhibiting RNase attenuators (KIRAs such as KIRA6) allosterically inhibit IRE1α RNase activity through kinase domain binding; (3) PERK kinase inhibitors (GSK2606414, GSK2656157) prevent eIF2α phosphorylation and ATF4 induction. These combined strategies disrupt pro-angiogenic signaling and inhibit tumor progression.

proteostasis mechanisms has revealed therapeutic vulnerabilities, though clinical translation remains challenging.

Hypoxia-induced ER stress in tumors

Cancer cells face dual stressors—hypoxia and nutrient deprivation—that challenge protein homeostasis [14]. These stresses cause accumulation of misfolded proteins in the ER, triggering the unfolded protein response (UPR). While excessive ER stress can trigger apoptosis, cancer cells have evolved to exploit the UPR as a survival mechanism, creating "proteostasis addiction"—a dependence on chronically activated UPR pathways for survival and proliferation [25,29].

In the hypoxic tumor microenvironment, all three UPR branches (PERK, IRE1α, ATF6) are constitutively activated. Rather than inducing apoptosis, this chronic UPR activation promotes tumor growth through several mechanisms: enhanced angiogenesis through VEGF induction, metabolic reprogramming to support cancer cell survival, resistance to apoptosis through upregulation of anti-apoptotic proteins, and promotion of metastasis through regulation of epithelial-mesenchymal transition [29].

UPR-mediated tumor angiogenesis

An important mechanism linking tumor hypoxia, UPR activation, and tumor progression is the promotion of angiogenesis—the formation of new blood vessels that supply tumors with oxygen and nutrients [29]. This process represents a sophisticated crosstalk between hypoxic tumor cells and the surrounding vasculature.

Within hypoxic tumor cells, activation of PERK and IRE1α leads to expression of the transcription factors ATF4 and XBP1s. These factors bind to the VEGF promoter, inducing VEGF transcription and secretion. The released VEGF then acts as a paracrine signal, binding to VEGF receptors (VEGFR-1 and VEGFR-2) on nearby endothelial cells and initiating pro-angiogenic signaling cascades [29].

In the responding endothelial cells, VEGF receptor activation triggers multiple downstream pathways. The phosphatidylinositol 3-kinase-protein kinase B (PI3K-AKT) pathway promotes cell survival, proliferation, and migration—processes essential for new vessel formation. Concurrently, VEGF signaling activates mechanistic target of rapamycin complex 1 (mTORC1), which phosphorylates and activates the ER stress sensors ATF6 and PERK, establishing a feed-forward amplification loop [29]. These activated UPR sensors further enhance AKT phosphorylation through activation of mTORC2 (a distinct mTOR-containing complex), reinforcing endothelial cell survival and angiogenic capacity. The result is a self-perpetuating cycle: tumor-derived VEGF activates endothelial cell UPR signaling, which amplifies VEGF responsiveness, thereby driving robust angiogenesis and sustaining tumor growth [29].

Therapeutic targeting of the UPR in cancer

The recognition that cancer cells depend on chronic UPR signaling has spurred development of small-molecule inhibitors targeting each UPR branch. These agents aim to selectively kill cancer cells by pushing them past their proteostatic threshold into apoptosis, while sparing normal cells that maintain lower baseline UPR activity [25]. However, achieving this selectivity in clinical settings has proven difficult.

IRE1α inhibitors

IRE1α represents the most evolutionarily conserved UPR sensor and possesses both kinase and endoribonuclease (RNase) activities. Its RNase activity splices XBP1 mRNA to generate the active transcription factor XBP1s, which drives expression of genes supporting cancer cell survival [30].

Direct RNase inhibitors

Several compounds have been developed that directly bind to and inhibit IRE1α RNase activity. The pharmacophore salicylaldehyde contains a reactive electrophile that covalently binds to the IRE1α RNase active site. This scaffold has been used to develop multiple inhibitors including: (i) STF-083010, an early salicylaldehyde-based inhibitor that blocks XBP1 splicing and induces apoptosis in multiple myeloma cells. Limited by high required concentrations in some preclinical models; (ii) MKC-3946, An improved salicylaldehyde derivative with enhanced potency and selectivity for IRE1α RNase; demonstrated efficacy in hematological malignancies in preclinical studies; (iii) and 4μ8c, Another salicylaldehyde-based inhibitor that has shown efficacy in preclinical models of glioblastoma, breast cancer, and colorectal cancer [30]. These compounds have demonstrated anti-tumor effects in preclinical models, but clinical translation requires further validation of efficacy and safety profiles.

Kinase-inhibiting RNase attenuators (KIRAs)

An alternative approach targets the kinase domain of IRE1α. Inhibiting IRE1α kinase activity also suppresses its RNase function through allosteric effects [31]. KIRA6, a second-generation kinase-inhibiting RNase attenuator that binds to the ATP-binding pocket of the IRE1α kinase domain, inducing a conformation that allosterically inhibits RNase activity [31]. Beyond cancer, KIRA6 has demonstrated efficacy in protecting against retinal degeneration induced by ER stress in rodent models [32]. The advantage of KIRAs over direct RNase inhibitors is their potential for greater selectivity and reduced off-target effects [31,32]. However, their clinical utility in cancer treatment remains under investigation.

PERK inhibitors

PERK phosphorylates eIF2α, causing global translational repression while allowing selective translation of stress-responsive proteins including ATF4. In cancer, PERK signaling supports cell survival under hypoxic and nutrient-deprived conditions, making it a potential therapeutic target [33].

GSK2606414

The first selective PERK inhibitor developed, which binds to the PERK kinase domain and

Table 2. Summary of cancer therapeutics targeting proteostasis in hypoxic conditions

Target	Drug/Inhibitor	Mechanism of Action	Preclinical/Clinical Status	Key Findings	References
IRE1α RNase	STF-083010	Covalent RNase inhibition	Preclinical	Induces apoptosis in multiple myeloma; limited by high required concentration	[30]
IRE1α RNase	MKC-3946	Covalent RNase inhibition	Preclinical	Improved potency in hematological malignancies	[30]
IRE1α RNase	4μ8c	Covalent RNase inhibition	Preclinical	Efficacy in solid tumors (glioblastoma, breast, colorectal)	[30]
IRE1α kinase	KIRA6	Allosteric inhibition via kinase domain	Preclinical	Protects against retinal degeneration; potential in cancer	[31,32]
PERK kinase	GSK2606414	ATP-competitive kinase inhibition	Preclinical	CNS-penetrant; protects against prion disease; β-cell toxicity	[34]
PERK kinase	GSK2656157	ATP-competitive kinase inhibition	Preclinical	Anti-tumor efficacy in pancreatic cancer and multiple myeloma xenografts; β-cell toxicity	[33]

prevents eIF2α phosphorylation. GSK2606414 demonstrated oral bioavailability and central nervous system penetration, protecting against neurodegeneration in prion-infected mice by blocking toxic ATF4-CHOP signaling [34]. In cancer models, this compound showed anti-tumor activity, though further development was limited by toxicity concerns.

GSK2656157

An improved compound with enhanced selectivity and potency. Preclinical studies showed anti-tumor efficacy in pancreatic adenocarcinoma and multiple myeloma xenograft models implanted in immunodeficient mice [33]. GSK2656157 treatment reduced tumor growth, decreased angiogenesis, and sensitized tumors to chemotherapy in these models.

Challenges and limitations

Despite encouraging preclinical results, PERK inhibitors face a substantial challenge: pancreatic β-cell toxicity. PERK plays an essential role in β-cell function, and genetic or pharmacological PERK inhibition causes β-cell death and diabetes [33]. This has necessitated careful evaluation of dosing regimens, development of tumor-selective delivery methods, or identification of more selective inhibitors that spare pancreatic function while maintaining anti-tumor activity.

Alternative strategies under investigation include: (i) intermittent dosing schedules to allow β-cell recovery, (ii) combination therapies using lower PERK inhibitor doses with other agents, (iii) development of substrate-selective PERK inhibitors that block ATF4 induction without affecting other eIF2α-dependent processes, (iv) targeting downstream effectors of PERK signaling (e.g., ATF4 or CHOP) rather than PERK itself. The clinical utility of PERK inhibition in cancer treatment remains uncertain pending resolution of these toxicity issues.

ATF6 inhibitors

While less extensively studied than IRE1α and PERK, ATF6 also represents a potential therapeutic target. ATF6 activation requires proteolytic cleavage by site-1 and site-2 proteases in the Golgi. Inhibitors of these proteases or compounds that prevent ATF6 translocation to the Golgi are under investigation but remain in early preclinical development [25]. The therapeutic window and potential toxicities of ATF6 inhibition in cancer treatment are not yet well-defined.

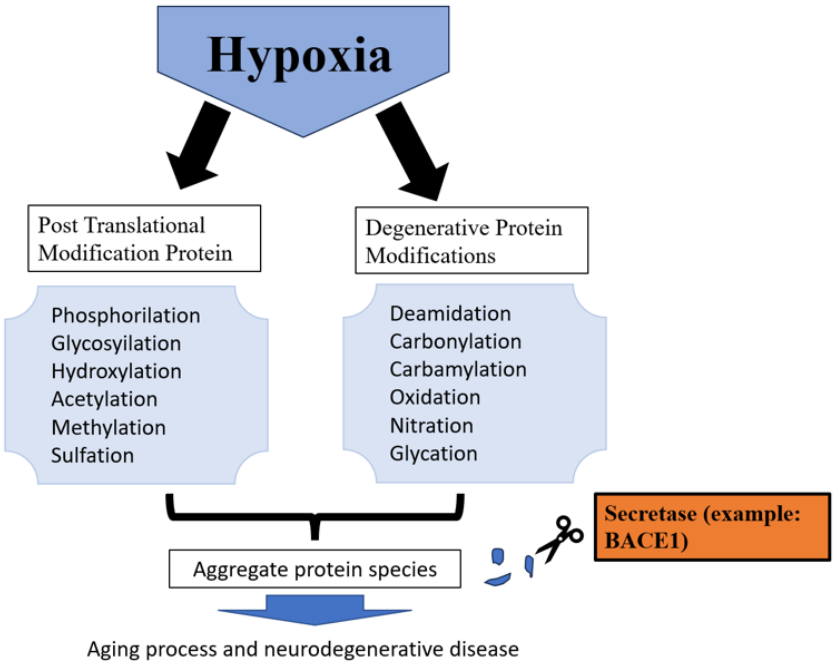

Figure 5. Strategies for eliminating protein aggregates in aging and neurodegenerative diseases. Multiple complementary approaches target protein aggregate clearance and prevention. (1) Reducing aggregate formation: γ-secretase modulators and BACE1 inhibitors reduce production of amyloid β-protein from APP processing; α-secretase activators promote non-amyloidogenic APP processing. (2) Enhancing degradation: Autophagy activators (rapamycin analogs, trehalose) and proteasome activators increase clearance of aggregated proteins. (3) Promoting disaggregation: Chaperone upregulation through HSF1 activation enhances protein refolding capacity; disaggregase activation (HSP70-HSP40 systems) dissolves existing aggregates. (4) Preventing aggregation: Small molecule inhibitors that bind amyloidogenic proteins prevent fibril formation; immunotherapy using antibodies targets aggregated species for microglial clearance. These integrated strategies aim to restore proteostasis balance and slow neurodegenerative progression.

Neurodegenerative diseases: clearing toxic protein aggregates

Hypoxia and neurodegeneration

Conditions that impair cerebral blood flow and oxygen delivery—including stroke, vascular dementia, chronic hypoxemia, and sleep apnea—lead to acute or chronic brain hypoxia, contributing to cognitive decline and neurodegeneration [35]. Clinical and experimental evidence supports that cerebrovascular disease and hypoxic-ischemic brain injury are major contributors to cognitive impairment and dementia [35].

Hypoxia affects the brain through mechanisms beyond simple energy depletion. Oxygen deprivation alters gene expression patterns, impairs synaptic function, and affects protein homeostasis through post-translational modifications [36]. These protein modifications accumulate over time, contributing to age-related cognitive decline and neurodegenerative diseases.

Protein modifications and aggregate formation

Reactive oxygen species (ROS) generated during hypoxia and aging cause extensive oxidative protein modifications, including carbonylation, glycation (formation of advanced glycation end products or AGEs), oxidation of specific amino acids, deamidation, lipoxidation (modification by lipid peroxidation products), and citrullination [36]. These modifications alter protein charge, hydrophobicity, and structure, promoting protein misfolding and aggregation.

Amyloidosis—the pathological accumulation of protein aggregates in tissues—is a defining feature of most neurodegenerative diseases. Different diseases are characterized by accumulation of distinct amyloidogenic proteins: Alzheimer's disease (amyloid β-protein plaques and tau neurofibrillary tangles), Parkinson's disease (α-synuclein Lewy bodies), Huntington's disease (mutant huntingtin protein aggregates), amyotrophic lateral sclerosis

(SOD1, TDP-43, and FUS aggregates), and Creutzfeldt-Jakob disease (misfolded prion protein aggregates).

These protein aggregates sequester functional proteins, impair proteasome and autophagy function, disrupt axonal transport, trigger inflammatory responses, and induce synaptic dysfunction and neuronal death. Hypoxia accelerates this pathology by increasing ROS production, which enhances protein oxidation and cross-linking, promotes conversion of soluble proteins into aggregation-prone forms, and creates a cellular environment conducive to amyloid formation [36].

Therapeutic strategies: reducing aggregate formation

Given the central role of protein aggregation in neurodegeneration, therapeutic strategies have focused on preventing aggregate formation by reducing production of amyloidogenic proteins. For Alzheimer's disease, the most advanced approaches target secretases—enzymes that proteolytically process the amyloid precursor protein (APP). However, clinical outcomes have been disappointing despite strong mechanistic rationale.

APP processing pathways

APP is a transmembrane protein that undergoes sequential proteolytic cleavage by secretase enzymes through two distinct pathways. The non-amyloidogenic pathway involves α-secretase cleaving within the Aβ sequence, preventing Aβ formation. The amyloidogenic pathway involves β-secretase (BACE1) cleaving APP at the N-terminus of Aβ, followed by γ-secretase cleaving the transmembrane domain to release Aβ peptides. Aβ42 is particularly prone to aggregation and is the primary component of amyloid plaques [37].

γ-secretase modulators (GSMs)

γ-Secretase is a multi-subunit protease complex composed of presenilin, nicastrin, APH-1, and PEN-2. This complex cleaves numerous substrates beyond APP, including Notch receptors essential for development and adult tissue homeostasis [37].

First-generation γ-secretase inhibitors completely blocked enzyme activity, reducing total Aβ production. However, clinical trials were terminated due to severe adverse effects including cognitive worsening, skin cancers, and gastrointestinal toxicity—consequences of Notch signaling inhibition [37].

Second-generation γ-secretase modulators (GSMs) subtly alter enzyme activity without completely blocking it. GSMs shift the cleavage pattern to favor production of shorter, less aggregation-prone Aβ peptides over longer, aggregation-prone forms without affecting Notch processing [37]. Several non-steroidal anti-inflammatory drugs (NSAIDs) were found to possess GSM activity, leading to development of more potent, selective GSM compounds. These agents showed encouraging results in preclinical models, reducing Aβ42 levels and plaque formation without Notch-related toxicity [37]. However, clinical translation has been limited, with no GSMs achieving regulatory approval to date.

BACE1 inhibitors

BACE1 (β-site APP cleaving enzyme 1) represents the rate-limiting enzyme in Aβ production, making it a potential therapeutic target [38]. Unlike γ-secretase, BACE1 has a more limited substrate repertoire, suggesting that its inhibition might produce fewer adverse effects.

Drug development challenges

BACE1 inhibitor development faced substantial challenges including blood-brain barrier penetration requirements and the need for highly potent inhibitors due to high concentrations of APP at neuronal membranes [38]. Despite these obstacles, several BACE1 inhibitors advanced to Phase III clinical trials in the mid-to-late 2010s.

Clinical trial failures

Between 2017 and 2019, all major BACE1 inhibitor Phase III trials were discontinued due to lack of efficacy. Verubecestat (Merck) trials in both mild-to-moderate AD and prodromal AD

patients showed no cognitive benefit and trends toward cognitive worsening [39,40]. Atabecestat (Janssen/Shionogi) development was halted due to liver enzyme elevations and cognitive decline in treated patients [41]. Lanabecestat (Eli Lilly/AstraZeneca) and elenbecestat (Eisai/Biogen) trials were similarly terminated for futility or safety concerns [42,43].

Notably, these trials successfully reduced cerebrospinal fluid (CSF) and brain Aβ levels, confirming target engagement. The disconnect between biochemical efficacy (Aβ reduction) and clinical efficacy (cognitive outcomes) has raised fundamental questions about therapeutic strategies for Alzheimer's disease [44].

The BACE1 inhibitor failures have prompted extensive re-evaluation of Alzheimer's disease therapeutic approaches. Multiple interpretations have been proposed, and the field has not reached consensus on their relative importance:

a. Timing hypothesis

One prevailing hypothesis is that therapeutic interventions must begin earlier in disease progression, before irreversible neuronal damage occurs [44,45]. Autopsy studies demonstrate that substantial neuronal loss has already occurred by the time patients receive clinical diagnoses [46]. Amyloid imaging studies show that plaques accumulate 15-20 years before symptom onset [47].

However, this interpretation faces challenges. Verubecestat trials enrolled patients with prodromal AD (very early symptomatic disease), yet still failed to demonstrate benefit [40]. This suggests that either intervention must occur even earlier (in presymptomatic individuals), or that factors beyond timing explain the failures.

b. Target validity questions

The failures have intensified debate about the amyloid cascade hypothesis. While amyloid accumulation clearly associates with AD and genetic evidence (APP, PSEN1, PSEN2 mutations) supports a causal role, the consistent failure of anti-amyloid therapies to improve cognition raises questions [48]. Some researchers argue that amyloid may be a consequence or epiphenomenon rather than the primary driver of neurodegeneration, or that it plays a role only in disease initiation but not progression [49].

c. Mechanism-based toxicity

BACE1 cleaves multiple substrates beyond APP, including neuregulin-1 (important for myelination), seizure protein 6 family members (involved in synaptic function), and voltage-gated sodium channel β-subunits [50]. Complete or near-complete BACE1 inhibition may disrupt these normal physiological processes, potentially offsetting any benefits from Aβ reduction. BACE1 knockout mice exhibit hypomyelination, schizophrenia-like behaviors, and axon guidance defects, supporting this concern [51].

The observation that higher doses of BACE1 inhibitors produced worse cognitive outcomes in some trials is consistent with mechanism-based toxicity [39]. This suggests that BACE1 may not be a viable therapeutic target regardless of timing, or that partial inhibition strategies need to be developed.

d. Disease complexity and multi-factorial pathogenesis

Alzheimer's disease involves multiple pathological processes beyond amyloid accumulation, including tau aggregation and spread, chronic neuroinflammation, synaptic dysfunction, mitochondrial impairment, and vascular contributions [52]. Tau pathology correlates more strongly with cognitive decline than amyloid burden [53]. Single-target therapies addressing only amyloid may be insufficient to alter disease trajectory, particularly in late-stage disease where multiple pathogenic processes are active.

e. Patient heterogeneity

Recent evidence suggests that AD is a heterogeneous syndrome with multiple subtypes that may have different underlying mechanisms [54]. Some patients

may have predominantly amyloid-driven disease, while others have tau-predominant, inflammatory, or metabolic variants. Clinical trials that do not stratify patients by disease subtype may dilute treatment effects in responsive subgroups.

Implications for future therapeutic development

These considerations have important implications for AD drug development: (i) Trials are increasingly enrolling presymptomatic individuals with biomarker evidence of amyloid accumulation, though this approach raises ethical challenges of treating healthy individuals and requires long trial durations of 10+ years to clinical endpoints [55]. (ii) Increased focus on tau, neuroinflammation, synaptic protection, and metabolic dysfunction as therapeutic targets [56]. (iii) Recognition that multi-target approaches addressing multiple pathological processes simultaneously may be necessary [57]. (iv) Development of biomarker-based patient stratification to identify subgroups most likely to benefit from specific interventions [58]. (v) For BACE1 and similar targets with physiological functions, strategies that partially reduce activity while preserving essential functions may be safer than complete inhibition [50]. The path forward for AD therapeutics remains uncertain. While mechanistic understanding has advanced substantially, translating this knowledge into effective treatments continues to be a major challenge in neuroscience and medicine.

Conclusion

Understanding proteostasis networks under hypoxic conditions reveals how disruptions in protein synthesis, folding, quality control, or degradation cascade into broader proteome imbalance and cellular dysfunction. Hypoxia increases reactive oxygen species levels, inducing protein oxidation and ER stress that triggers UPR signaling and promotes toxic protein aggregate accumulation, contributing to cancer progression and neurodegenerative disease pathogenesis. Disease-specific vulnerabilities enable targeted therapeutic strategies: cancer cells' addiction to chronic UPR signaling can be exploited through IRE1α RNase inhibitors, kinase-inhibiting RNase attenuators, and PERK kinase inhibitors to disrupt tumor survival, while neurons vulnerable to aggregate accumulation may benefit from enhanced degradation via autophagy activation and chaperone upregulation. Success will likely require integrated approaches combining reduced production of aggregation-prone proteins, enhanced chaperone capacity, improved degradation pathways, and oxidative stress protection, with early intervention proving essential as trials in symptomatic neurodegenerative patients have largely failed. The field now stands at an exciting juncture where basic science has identified key mechanisms and therapeutic targets; the challenge lies in translating this knowledge into effective clinical interventions that restore proteome balance and improve outcomes for patients with cancer, neurodegenerative diseases, and other proteostasis-related disorders.

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Declaration of interest

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References

1. Kim YE, Hipp MS, Bracher A, Hayer-Hartl M, Hartl FU. Molecular chaperone functions in protein folding and proteostasis. Annu Rev Biochem. 2013;82: 323-355. https://doi.org/10.1146/annurev-biochem-060208-092442
2. Hipp MS, Kasturi P, Hartl FU. The proteostasis network and its decline in ageing. Nat Rev Mol Cell Biol. 2019;20: 421-435. https://doi.org/10.1038/s41580-019-0101-y
3. Balchin D, Hayer-Hartl M, Hartl FU. In vivo aspects of protein folding and quality control. Science (1979). 2016;353: aac4354. https://doi.org/10.1126/science.aac4354
4. Alberti S, Hyman AA. Biomolecular condensates at the nexus of cellular stress, protein aggregation disease and ageing. Nat Rev Mol Cell Biol. 2021;22: 196-213. https://doi.org/10.1038/s41580-020-00326-6
5. Badawi Y, Shi H. Relative contribution of prolyl hydroxylase-dependent and -independent degradation of HIF-1alpha by proteasomal pathways in cerebral ischemia. Front Neurosci. 2017;11: 239. https://doi.org/10.3389/fnins.2017.00239
6. Nelson D, Cox M. Lehninger Biochemie. 3rd ed. Berlin, Germany: Springer; 2005.
7. Kulak NA, Geyer PE, Mann M. Loss-less nano-fractionator for high sensitivity, high coverage proteomics. Mol Cell Proteomics. 2017;16: 694-705. https://doi.org/10.1074/mcp.O116.065136
8. Gomez-Pastor R, Burchfiel ET, Thiele DJ. Regulation of heat shock transcription factors and their roles in physiology and disease. Nat Rev Mol Cell Biol. 2018;19: 4-19. https://doi.org/10.1038/nrm.2017.73
9. Li Y, Li S, Wu H. Ubiquitination-Proteasome System (UPS) and Autophagy Two Main Protein Degradation Machineries in Response to Cell Stress. Cells. 2022;11: 851. https://doi.org/10.3390/cells11050851
10. Fregno I, Molinari M. Proteasomal and lysosomal clearance of faulty secretory proteins: ER-associated degradation (ERAD) and ER-to-lysosome-associated degradation (ERLAD) pathways. Crit Rev Biochem Mol Biol. 2019;54: 153-163. https://doi.org/10.1080/10409238.2019.1610351
11. Robinson PJ, Bulleid NJ. Mechanisms of Disulfide Bond Formation in Nascent Polypeptides Entering the Secretory Pathway. Cells. 2020;9: 1994. https://doi.org/10.3390/cells9091994
12. Penke B, Bogár F, Crul T, Sántha M, Tóth ME, Vígh L. Heat Shock Proteins and Autophagy Pathways in Neuroprotection: From Molecular Bases to Pharmacological Interventions. Int J Mol Sci. 2018;19: 325. https://doi.org/10.3390/ijms19010325
13. Woerner AC, Frottin F, Hornburg D, Feng LR, Meissner F, Patra M, et al. Cytoplasmic protein aggregates interfere with nucleocytoplasmic transport of protein and RNA. Science (1979). 2016;351: 173-176. https://doi.org/10.1126/science.aad2033
14. Balch WE, Morimoto RI, Dillin A, Kelly JW. Adapting proteostasis for disease intervention. Science (1979). 2008;319: 916-919. https://doi.org/10.1126/science.1141448
15. Kaufman DM, Wu X, Scott BA, Itani OA, Van Gilst MR, Bruce JE, et al. Ageing and hypoxia cause protein aggregation in mitochondria. Cell Death Differ. 2017;24: 1730-1738. https://doi.org/10.1038/cdd.2017.101
16. Agrawal A, Rathor R, Suryakumar G. Oxidative protein modification alters proteostasis under acute hypobaric hypoxia in skeletal muscles: a comprehensive in vivo study. Cell Stress Chaperones. 2017;22: 429-443. https://doi.org/10.1007/s12192-017-0795-8
17. Díaz-Villanueva J, Díaz-Molina R, García-González V. Protein Folding and Mechanisms of Proteostasis. Int J Mol Sci. 2015;16: 17193-17230. https://doi.org/10.3390/ijms160817193
18. Sučec I, Bersch B, Schanda P. How do Chaperones Bind (Partly) Unfolded Client Proteins? Front Mol Biosci. 2021;8. https://doi.org/10.3389/fmolb.2021.762005
19. Teodoro RO, O'Farrell PH. Nitric oxide-induced suspended animation promotes survival during hypoxia. EMBO J. 2003;22: 580-587. https://doi.org/10.1093/emboj/cdg070
20. Koumenis C, Naczki C, Koritzinsky M, Rastani S, Diehl A, Sonenberg Nahum and Koromilas A, et al. Regulation of protein synthesis by hypoxia via activation of the endoplasmic reticulum kinase PERK and phosphorylation of the translation initiation factor eIF2alpha. Mol Cell Biol. 2002;22: 7405-7416. https://doi.org/10.1128/MCB.22.21.7405-7416.2002
21. Gromley C. Characterization of neuronal specific responses to induced misfolded protein stress in Caenorhabditis elegans. James Madison University. 2017.
22. Wen Y, Hu J, Wang J, Liu X, Li S, Luo Y. Effect of glycolysis and heat shock proteins on hypoxia adaptation of Tibetan sheep at different altitude. Gene. 2021;803: 145893. https://doi.org/10.1016/j.gene.2021.145893
23. Xu H. Cochaperones enable Hsp70 to use ATP energy to stabilize native proteins out of the folding equilibrium. Sci Rep. 2018;8: 13213. https://doi.org/10.1038/s41598-018-31641-w
24. Chaudhary P, Suryakumar G, Prasad R, Singh SN, Ali S, Ilavazhagan G. Effect of acute hypobaric hypoxia on skeletal muscle protein turnover . Al Ameen Journal Medical Science. 2012;5: 355-361.
25. Oakes SA. Endoplasmic reticulum proteostasis: a key checkpoint in cancer. American Journal of Physiology-Cell Physiology. 2017;312: C93-C102. https://doi.org/10.1152/ajpcell.00266.2016
26. Sano R, Reed JC. ER stress-induced cell death mechanisms. Biochimica et Biophysica Acta (BBA) - Molecular Cell Research. 2013;1833: 3460-3470. https://doi.org/10.1016/j.bbamcr.2013.06.028

27. Shimoda LA, Undem C. Interactions between calcium and reactive oxygen species in pulmonary arterial smooth muscle responses to hypoxia. Respir Physiol Neurobiol. 2010;174: 221-229. https://doi.org/10.1016/j.resp.2010.08.014
28. Bartoli M, Richard I. Calpains in muscle wasting. Int J Biochem Cell Biol. 2005;37: 2115-2133. https://doi.org/10.1016/j.biocel.2004.12.012
29. Dufey E, Urra H, Hetz C. ER proteostasis addiction in cancer biology: Novel concepts. Semin Cancer Biol. 2015;33: 40-47. https://doi.org/10.1016/j.semcancer.2015.04.003
30. Cross BCS, Bond PJ, Sadowski PG, Jha BK, Zak J, Goodman JM, et al. The molecular basis for selective inhibition of unconventional mRNA splicing by an IRE1-binding small molecule. Proc Natl Acad Sci U S A. 2012;109: E869-E878. https://doi.org/10.1073/pnas.1115623109
31. Wang L, Perera BGK, Hari SB, Bhhatarai B, Backes BJ, Seeliger MA, et al. Divergent allosteric control of the IRE1α endoribonuclease using kinase inhibitors. Nat Chem Biol. 2012;8: 982-989. https://doi.org/10.1038/nchembio.1094
32. Ghosh R, Wang L, Wang ES, Perera BGK, Igbaria A, Morita S, et al. Allosteric inhibition of the IRE1α RNase preserves cell viability and function during endoplasmic reticulum stress. Cell. 2014;158: 534-548. https://doi.org/10.1016/j.cell.2014.07.002
33. Atkins C, Liu Q, Minthorn E, Zhang S-Y, Figueroa DJ, Moss K, et al. Characterization of a novel PERK kinase inhibitor with antitumor and antiangiogenic activity. Cancer Res. 2013;73: 1993-2002. https://doi.org/10.1158/0008-5472.CAN-12-3109
34. Moreno JA, Halliday M, Molloy C, Radford H, Verity N, Axten JM, et al. Oral Treatment Targeting the Unfolded Protein Response Prevents Neurodegeneration and Clinical Disease in Prion-Infected Mice. Sci Transl Med. 2013;5. https://doi.org/10.1126/scitranslmed.3006767
35. Kalaria RN, Ihara M. Dementia: Vascular and neurodegenerative pathways-will they meet? Nat Rev Neurol. 2013;9: 487-488. https://doi.org/10.1038/nrneurol.2013.164
36. Adav SS, Sze SK. Hypoxia-induced Degenerative Protein Modifications associated with aging and age-associated disorders. Aging Dis. 2020;11: 341-364. https://doi.org/10.14336/AD.2019.0604
37. Wolfe MS. γ-Secretase as a Target for Alzheimer's Disease. Current State of Alzheimer's Disease Research and Therapeutics. Elsevier; 2012. pp. 127-153. https://doi.org/10.1016/B978-0-12-394816-8.00004-0
38. Zhao J, Fu Y, Yasvoina M, Shao P, Hitt B, O'Connor T, et al. β-Site Amyloid Precursor Protein Cleaving Enzyme 1 Levels Become Elevated in Neurons around Amyloid Plaques: Implications for Alzheimer's Disease Pathogenesis. The Journal of Neuroscience. 2007;27: 3639-3649. https://doi.org/10.1523/JNEUROSCI.4396-06.2007
39. Egan MF, Kost J, Tariot PN, Aisen PS, Cummings JL, Vellas B, et al. Randomized trial of verubecestat for mild-to-moderate Alzheimer's disease. N Engl J Med. 2018;378(18):1691-1703. https://doi.org/10.1056/NEJMoa1706441
40. Egan MF, Kost J, Voss T, Mukai Y, Aisen PS, Cummings JL, et al. Randomized trial of verubecestat for prodromal Alzheimer's disease. N Engl J Med. 2019;380(15):1408-1420. https://doi.org/10.1056/NEJMoa1812840
41. Henley DB, Sundell KL, Sethuraman G, Dowsett SA, May PC. Safety profile of semagacestat, a gamma-secretase inhibitor: IDENTITY trial findings. Curr Med Res Opin. 2014;30(10):2021-2032. https://doi.org/10.1185/03007995.2014.939167
42. Moussa-Pacha NM, Abdin SM, Omar HA, Alniss H, Al-Tel TH. BACE1 inhibitors: Current status and future directions in treating Alzheimer's disease. Med Res Rev. 2020;40(1):339-384. https://doi.org/10.1002/med.21622
43. Mullard A. BACE failures lower AD expectations, again. Nat Rev Drug Discov. 2018;17(6):385. https://doi.org/10.1038/nrd.2018.94
44. Panza F, Lozupone M, Logroscino G, Imbimbo BP. A critical appraisal of amyloid-β-targeting therapies for Alzheimer disease. Nat Rev Neurol. 2019;15(2):73-88. https://doi.org/10.1038/s41582-018-0116-6
45. Sperling RA, Aisen PS, Beckett LA, Bennett DA, Craft S, Fagan AM, et al. Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 2011;7(3):280-292. https://doi.org/10.1016/j.jalz.2011.03.003
46. Gomez-Isla T, Hollister R, West H, Mui S, Growdon JH, Petersen RC, et al. Neuronal loss correlates with but exceeds neurofibrillary tangles in Alzheimer's disease. Ann Neurol. 1997;41(1):17-24. https://doi.org/10.1002/ana.410410106
47. Jack CR Jr, Knopman DS, Jagust WJ, Petersen RC, Weiner MW, Aisen PS, et al. Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. Lancet Neurol. 2013;12(2):207-216. https://doi.org/10.1016/S1474-4422(12)70291-0
48. Karran E, De Strooper B. The amyloid cascade hypothesis: are we poised for success or failure? J Neurochem. 2016;139 Suppl 2:237-252. https://doi.org/10.1111/jnc.13632
49. Morris GP, Clark IA, Vissel B. Questions concerning the role of amyloid-β in the definition, aetiology and diagnosis of Alzheimer's disease. Acta Neuropathol. 2018;136(5):663-689. https://doi.org/10.1007/s00401-018-1918-8
50. Yan R, Vassar R. Targeting the β secretase BACE1 for Alzheimer's disease therapy. Lancet Neurol. 2014;13(3):319-329. https://doi.org/10.1016/S1474-4422(13)70276-X

51. Hu X, Hicks CW, He W, Wong P, Macklin WB, Trapp BD, et al. Bace1 modulates myelination in the central and peripheral nervous system. Nat Neurosci. 2006;9(12):1520-1525. https://doi.org/10.1038/nn1797

52. De Strooper B, Karran E. The cellular phase of Alzheimer's disease. Cell. 2016;164(4):603-615. https://doi.org/10.1016/j.cell.2015.12.056

53. Nelson PT, Alafuzoff I, Bigio EH, Bouras C, Braak H, Cairns NJ, et al. Correlation of Alzheimer disease neuropathologic changes with cognitive status: a review of the literature. J Neuropathol Exp Neurol. 2012;71(5):362-381. https://doi.org/10.1097/NEN.0b013e31825018f7

54. Ferreira D, Nordberg A, Westman E. Biological subtypes of Alzheimer disease: A systematic review and meta-analysis. Neurology. 2020;94(10):436-448. https://doi.org/10.1212/WNL.0000000000009058

55. Sperling RA, Rentz DM, Johnson KA, Karlawish J, Donohue M, Salmon DP, et al. The A4 study: stopping AD before symptoms begin? Sci Transl Med. 2014;6(228):228fs13. https://doi.org/10.1126/scitranslmed.3007941

56. Cummings J, Lee G, Ritter A, Sabbagh M, Zhong K. Alzheimer's disease drug development pipeline: 2020. Alzheimers Dement (N Y). 2020;6(1):e12050. https://doi.org/10.1002/trc2.12050

57. Cummings J, Ritter A, Zhong K. Clinical trials for disease-modifying therapies in Alzheimer's disease: a primer, lessons learned, and a blueprint for the future. J Alzheimers Dis. 2018;64(s1):S3-S22. https://doi.org/10.3233/JAD-179901

58. Hampel H, Toschi N, Babiloni C, Baldacci F, Black KL, Bokde ALW, et al. Revolution of Alzheimer precision neurology. Passageway of systems biology and neurophysiology. J Alzheimers Dis. 2018;64(s1):S47-S105. https://doi.org/10.3233/JAD-179932