university of groningen / faculty of science and engineering

UNIVERSITY OF GRONINGEN

Faculty of Science and Engineering

umcg

UMCG Department: Cell Biology

Research Group: Steven Bergink Group

HSP-70 & HSP-90: role in neurodegenerative diseases and potential as therapeutic targets.

Molecular Biology & Biotechnology MSc - ESSAY

STUDENT: Alejandro Prats Luján (*S3044777*)

SUPERVISOR: Steven Bergink

GRONINGEN, JUNE 2018

ABSTRACT

Neurodegenerative diseases have been increasing for the past decades, and will continue their rise in the incoming years. This manifests the importance to develop effective therapies. Despite their differences, all diseases have in common the protein aggregation and dysregulation of the Proteostasis. One of the main regulators in protein homeostasis are the heat-shock proteins (HSP), assisting the protein folding as chaperones. Within this group of molecules, HSP-70 and HSP-90 have become a target for many therapeutic approaches by inhibition or induction of their activity. Additional therapies have targeted other molecules involved in their regulation such as the transcription factor HSF-1 or histone deacetylates (HDAC), and others have focused in repairing mutated genes or delivering molecules with chaperone properties. Many of these therapeutic approaches have shown promising results and entered their final steps of the clinical trials. On the other hand, other approaches also showed promising applications, but failed to deliver the expectations or encountered toxicity and safety problems.

Many advances have been done in the past years, but many issues remain unsolved. Heat-shock proteins, and specially HSP-70 and HSP-90 families have proved to be an interesting target for therapeutic techniques, with further alternatives arising from the development of new and better techniques.

Neurodegenerative diseases, heat-shock proteins, HSP-70, HSP-90, therapeutic target

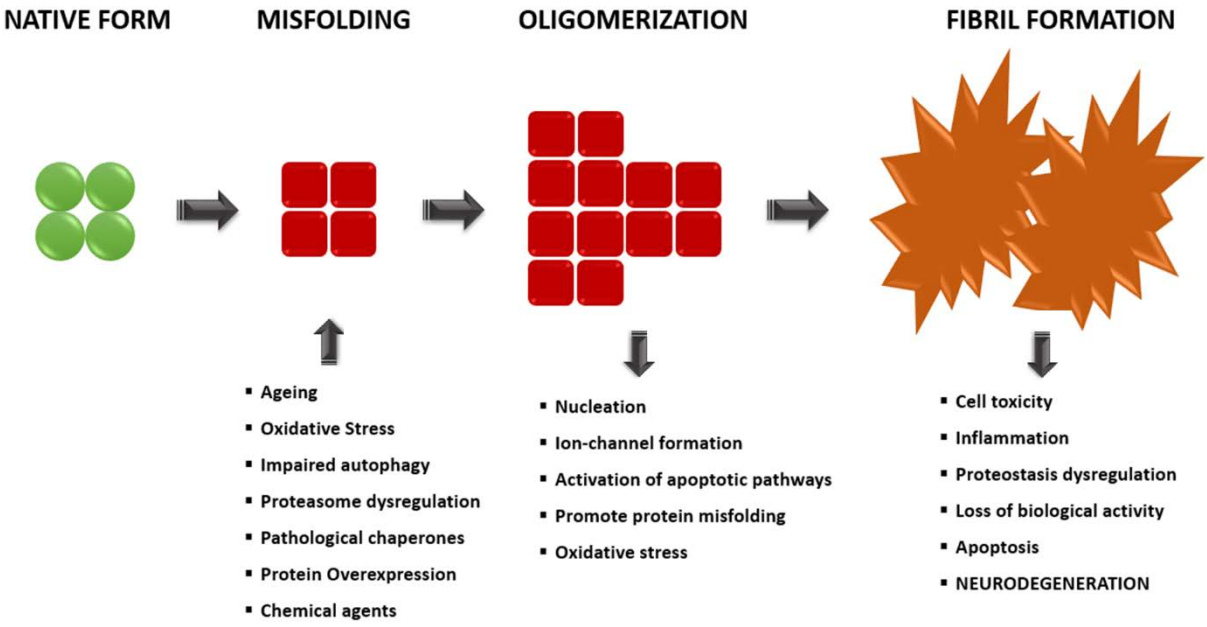
INDEX

Nowadays, 16% of the European population is over 65 years old, and is expected to increase to 25% by the year 2030. With aging comes the conditions that still today remain untreatable, representing one of the major medical challenges. In addition to the personal impact, these disorders have a big social and economic influence, costing around €130 billion per year, with few to none treatments focused only in palliating symptoms instead of tackling the cause (1,2). The pathology of neurodegenerative disorders is intriguing, as different conditions selectively damage different neuronal populations. These differences determine the clinical symptoms that are related to each disease. For example, damage in the hippocampus and the cerebral cortex is usually related to Alzheimer. In the case of Parkinson, the main areas affected are the hypothalamus and substantia nigra (3). Another feature is that most of these neurodegenerative diseases are genetically complex and some can appear sporadically, with the exception of Huntington disease where is inherited as monogenic (autosomal dominant) (3,4). The fact that some of them can be sporadic increases the difficulty to diagnose the disease in its early stages, as there is not a clear feature to identify the development of the disease. This manifests the need to identify markers for neurodegeneration prior the appearance of the first traditional symptoms. Nonetheless, one key factor that neurodegenerative diseases share in common is that their progression is usually associated with the generation of abnormal protein aggregates.

PROTEIN AGGREGATION IN NEURODEGENERATIVE DISEASES

Protein aggregation is a common physiological process in mammals required for some vital cellular functions. However, the aggregation of abnormal proteins can lead to the development of several diseases, known as amyloidosis. These diseases can either be systemic and affect the whole organism or can affect an specific organ, like in type 2 diabetes or Alzheimer. Protein aggregates can accumulate in different compartments within the system, some will create cytoplasmic inclusion bodies (Parkinson), some will accumulate extracellularly (prion-like diseases) and others will have both intracellular and extracellular accumulation (Alzheimer or Huntington) (5,6). Furthermore, protein aggregates can also differ in their morphology and composition, as some might be ordered as fibrils and other can lead to amorphous aggregates. Covalent modifications on the protein and mutations on the encoding gene will have an influence in forming different fibril structures and oligomers, and therefore influencing their toxicity. They might also include other elements such as chaperones, glycosaminoglycans (GAGs), proteoglycans (PGs), ubiquitylated proteins and subunits from the 26S proteasome complex (2,3,6). Many elements have a role in the development of these protein aggregates: the genetic background, biological agents (pathological chaperones), chemical agents (pesticides) and other soluble naturally occurring

proteins (6,7). Although the protein misfolding can be a critical factor for the development of the disease, they are not always the trigger as some proteins need further processing before initiating the aggregation (8). Furthermore, the protein deposition usually triggers other undesired mechanisms such as oxidative stress, immune system recruitment (inflammation), cell injury and finally apoptosis (5,9). The discovery and study of all these factors involved in the pathology of the diseases lead to the amyloid cascade hypothesis (2,9). Experimental evidence showed that the development of amyloid fibrils from soluble monomers is result of a complex multistep mechanism, in which several intermediate species of proteins interact with each other (2,10). These low molecular soluble oligomers are thought to be the most toxic species as they can catalyze the formation of more toxic species and bigger protein aggregates (2,9,10). Features of these soluble proteins allow them to start the nucleation process of protein aggregation and promote interactions between abnormal proteins and insoluble fibrils. The nucleation mechanism begins with the misfolding of soluble oligomers that will exposure their hydrophobic surface (normally hidden in the folded protein core). Differences in the structure and toxicity of the aggregates will be a result from a different content of hydrophobic residues (10). Several studies showed that in certain circumstances, aromatic residues might have a role in the fibrillization process and formation of amyloid aggregates (11). The attractive forces from the hydrophobic residues are very reactive and metastable, and will eventually lead into forming protofibrils by monomer addition. This can be further enhanced by a second nucleation process, such as fibril fragmentation (3,5,7,9) (*Picture 1*).



Picture 1. Scheme of the protein aggregation process. Causes and consequences. The process begins with the misfolding of the monomer and exposing its hydrophobic core. Interactions between misfolded proteins will lead to their oligomerization, and afterwards to the formation of larger fibrils. Several factors can cause the misfolding: ageing, pathological chaperones, chemical agents… Oligomerization and fibril formation will have an impact on cell homeostasis and trigger other mechanisms.

Several concepts have been developed as a result of this. One is that oligomeric intermediates are the molecules responsible for the pathogenesis, and exist in a kinetic equilibrium between the soluble monomeric state and the insoluble aggregated state (5). Another hypothesis relates the progression of the pathology with soluble monomers acting like prions. The donor cell will produce misfolded aggregates and propagate them to an acceptor cell, where the prion-like protein will interact with normal proteins and promote its modification (12,13). Recent studies have suggested that protein aggregates characteristic in Parkinson, Alzheimer and Huntington disorders might spread to unaffected areas such as in spongiform encephalopathies or kuru, and catalyze the deposition of new amyloid aggregates (5,12,13). However, more studies are needed to further corroborate the theory of pathology dispersion through prion-like molecules.

QUALITY CONTROL MECHANISMS: HOMEOSTASIS AND DYSREGULATION

As proteins have a tendency to form aggregates, cells have developed powerful quality control mechanisms to prevent dysfunctions and preserve the homeostasis (5). These mechanisms are in charge of keeping proteins in their soluble state and promote the degradation of those with aberrant structures. The quality control system is comprised of a variety of molecular components such as chaperones (also known as heat-shock proteins, HSP), the ubiquitin-proteasome system, autophagic degradation mechanisms and other trafficking and stress response pathways (5,6,14–16). Under normal conditions, the unfolded protein response (UPR) monitors the status in the endoplasmic reticulum (ER) where most proteins are folded. When the UPR remains active for a long time and homeostasis cannot be restored, it can serve as an apoptotic trigger that will eliminate the cell as a protective measure (15). The UPR detects misfolded proteins in the ER and then are exported to the cytosol, where they will go through two main pathways: one is the ubiquitin-proteasome system (UPS) that will recognize and process them for further degradation (ER-associated degradation, ERAD). Several studies proved that the proteasome system is highly regulated and can determine whether ubiquitylated proteins are degraded or recycled and survive, depending on the structural features of the protein (17). The other mechanism that proteins can encounter in the cytosol is the autophagy-lysosomal pathway in which more stable and long-term proteins are degraded (6). Despite their important role in preserving the homeostasis, a constant state of activation of the autophagy and protein degradation pathways can develop other issues such as mitochondrial stress, that will end up in cellular dysregulation. In addition, their efficiency upon aging is likely to decrease, with higher susceptibility to be overwhelmed and not fulfil their role (6,14).

HEAT-SHOCK PROTEINS: KEY REGULATORS OF CELLULAR HOMEOSTASIS

Heat-shock proteins are an heterogeneous group of molecules involved in the maintenance of cellular protein homeostasis. This highly conserved proteins can be classified into seven different families according to their molecular weight (8,16,18): HSP-70 (HSPA), the small HSPs (HSPB), HSP-90 (HSPC), HSP-100 (HSPH) and the chaperonins of HSP-60 (HSPD), HSP-10 (HSPE) and TCP-1 ring complex (TRiC). Most chaperones are induced under cellular stress like oxidative stress, hyperthermia, hypoxia and other types of chemical and physical stress. Their main role is to supervise and guide proteins from production to degradation, and guarantee the appropriate folding of the target proteins while avoiding its aggregation. Several papers have shown further roles in preventing the interaction of protein oligomers with cellular membranes, as well as promoting its assembly into less toxic larger species (19). Other study showed the effect of chaperones in the different phases of protein aggregation, having different roles whether is in the lag phase, elongation phase or the plateau phase. During the lag phase, native proteins misfold and start forming the more complex oligomers. In the elongation or growth phase this oligomers become larger, by nucleation processes and the addition of abnormal soluble proteins, to form the bigger and insoluble protofibrils. Finally in the saturation or plateau phase, the final fibril formation is completed. This studies showed that chaperones can prolong the lag phase and delay the primary and secondary nucleation, retaining the process in the early stages of soluble monomers (7,8,20). Nonetheless, chaperones also have other roles in the cell such as the stabilization of elements from the cytoskeleton and inhibition of apoptotic pathways (3,15,16). In all these processes, additional molecules have the role to assist chaperones. Known as co-chaperones, these molecules regulate the activity of their chaperones by affecting the recruitment, assisting the binding of other proteins and modulating the ATPase activity. They are also classified into different families such as the BAG-domain family (BAG1-6), DNAJ-domain family (comprising co-chaperone HSP-40 family) and the TPR-domain family (Hip, CHIP and Hop) (16,21).

Focusing on their homeostatic role, chaperones expression is induced by the accumulation of unfolded proteins and the involvement of the transcription factor HSF-1 (Heat-shock Factor 1). Both chaperones and the HSF-1 transcription factor interact as a complex to monitor the flux of misfolded proteins and intermediates, and coordinate several pathways of the Proteostasis Network (22). Under normal conditions, HSF-1 interacts with HSP-90 and remains as an inactive monomer in the cytosol. Its activation can be divided into four main steps: the process starts when cell stress is detected, then chaperones (HSP-90, HSP-70 and TRiC) are prevented to interact with HSF-1. Because of this, HSF-1 will change its inactive conformation (from monomer) to an active conformation capable of binding to DNA

(to trimer). In the next step, HSF-1 is translocated to the nucleus and targets the DNA to start the transcription. Two kinases (MEK and AMPK) have the role to promote or inhibit the translocation of HSF-1 into the nucleus. In the third step, HSF-1 triggers transcription under the influence of acetyltransferases and deacetylases that will act as promoters and suppressors of DNA binding activity of HSF-1. In the final step, E3 ubiquitin ligases will target HSF-1 in order to induce its degradation by the ubiquitin-proteasome system (22).

HSPA: THE HSP-70 FAMILY

As previously mentioned, the HSP-70 family is a major component of the protein quality control mechanisms. Members of this chaperone family are abundant in the cytosol as well as many organelles (23), and it has been demonstrated an additional role as regulator of protein aggregates in membrane-lacking subcompartments such as the ribosomes or nucleolus, where proteins can be synthesized (8). The mitochondrial HSP-70 (mtHsp70 or mortalin) has a major role in oxidative stress by regulation of the mitochondrial homeostasis, as well in the development of neurodegenerative diseases (16). Its structure is composed by two major domains: the nucleotide-binding domain (NBD) and the substrate-binding domain (SBD). The nucleotide-binding domain is in charge for binding ATP and its hydrolysis, although with very weak activity. This binding mechanism is under the regulation of co-chaperones such as DNAJ proteins and nucleotide exchange factors (NEFs). DNAJs can enhance the hydrolysis of ATP while NEFs promote the release of the resulting ADP. On the other hand, the substrate-binding domain contains the motif EEVD that binds to tetratricopeptide repeat domains (TRP), common in hydrophobic regions of proteins and polypeptides (23,24). Knowing its role in protein homeostasis, several studies have proved that HSP-70 is a critical component of the arsenal to fight the progress of neurodegenerative diseases, by interacting with the hydrophobic region of toxic oligomers and avoiding the aggregate formation (16). Because of this, the HSP-70 family has become a target for drug development, especially upregulation of their activity through its enhancement, or inhibition of other related molecules (HDAC or HSP-90). Some of the current therapeutic approaches are focused on the ATPase activity, by either competitive and noncompetitive inhibition mechanisms such as the compound VER-155008. Other compounds are focused on targeting the substrate-binding domain or the interactions with co-chaperones, like the compound 116-9e (23). More detailed information about chaperone-targeted therapies will be commented further in this report.

HSPC: THE HSP-90 FAMILY

Another molecular chaperone with a big role in protein homeostasis is HSP-90. Usually found as an homodimer with a V-shaped open conformation, its structure is composed by three domains: a 25 kDa N-terminal domain involved in the ATP binding and hydrolysis, a 40 kDa internal domain with the role of substrate peptide-binding site, and a 12 kDa C-terminal domain with the TPR-binding domain and the EEVD motif (23,25). As it happened with the HSP-70, this protein interacts with other co-chaperones that regulate its ATPase activity and conformations. It has been demonstrated its role as a scavenger protein in the development of Parkinson's Disease. It may act by shifting the aggregate's equilibrium towards more mature fibrils rather than the toxic oligomers (16). Because of this, HSP-90 has also become a target for the development of drugs and therapies. In the past years, many inhibitors have been reported and entered the clinical trials. As mentioned before, further information about therapies will be provided in the next points of the report.

THERAPIES TARGETING PROTEIN AGGREGATION

Several therapeutic strategies have been developed in the past decades to target protein aggregates and its formation, via *in vitro* studies and using animal models (5,6,26). Several difficulties arise in overcoming the protein aggregation: first is the limited knowledge on the molecular mechanism to target, and the possibility that more than one mechanism might be involved. Second is the development of an efficient drug candidate with little to none side effects. Related to it, these targets usually have physiological roles, therefore its inhibition or enhancement can produce unexpected side effects. Last one is the inability to mimic the human pathological condition in animal and *in vitro* models. This is the reason why many compounds are promising during the *in vitro* experiments but fail when further studied in animal models and clinical trials.

Nevertheless, many pharmaceutical compounds have been developed, some resulting in failure but many others entering the clinical trials with success. These compounds usually interact in a covalent or noncovalent way with several species from the aggregation pathway, and inhibit the formation of matured aggregates (2,5,6,8,19). The target of this inhibitors is to reduce the primary and secondary nucleation as well as the elongation, whenever they bind to oligomers or the matured fibrils respectively. Some examples include molecular chaperones (6,19,21,23,25,27,28), anti-inflammatory agents, antibodies, peptides and organic or inorganic compounds. The inhibition of BACE-1 and γ-secretase, or activation of α-secretase has become a potential therapeutic target and some compounds (CTS-21166, semagacesat and BMS-708163) reached the phase II and III of clinical trials (6). This effect is result of

inhibiting the two step sequential cleavage activity of BACE-1 and γ-secretase to create Aβ peptides from the amyloid precursor protein (APP). On the other hand, α-secretase can cleave within the Aβ peptide, avoiding its release and therefore its formation. Additionally, inhibition of the hyperphosphorylation of kinases involved in the aggregation process has also become a valid therapeutic target, especially in tauopathies (6,23). Another inhibitory compounds are focused in the depletion of the more toxic oligomers and protofibrilar species, and others have focused in redirecting these to form less toxic mature fibrils. Small compounds have been analyzed as inhibitors of aggregation such as polyphenols, phenothiazines and curcumins (2,6). Some studies analyzed how nanoparticles were able to sequester monomers and oligomers and avoid the fibril formation (29). As previously mentioned, chaperones and chaperone-like molecules (tafamidis) have shown to interact with oligomers and inhibit the formation of mature fibrils. On the other hand, inhibition of the aggregation mechanism can lead to higher concentrations of more toxic oligomers and therefore have a negative impact on the pathology. Because of this outcome, alternatives have been studied, such as the enhancement of the oligomer aggregation (6). This therapeutic approach is based on the formation of protein aggregates in order to reduce the concentration of more toxic intermediates (30). Therapies that do not target the protein aggregates have arisen from discoveries in the past years: the use of statins to reduce cholesterol showed to decrease neuronal protein aggregation (6). Another, is the use of immunotherapy to tackle prion-like pathologies, however with little success as high concentration of antibodies were required (5). One more is the use of metal chelators, as it has been shown that ions such a Cu, Zn and Fe can enhance the mechanism of protein aggregation (6).

HSP-70 & HSP-90 AS THERAPY TARGETS

HSPs (chaperones) and chaperone-like molecules have been gaining attention as targets for pharmaceutical therapies, with promising results (6,16,23,25). These compounds are mainly focused on the HSP-70 family and its mechanism, differentiating between those that act as inhibitors and those that have an enhancing effect, as well as other alternatives (*Picture 2*). They can be divided into different groups: modulators of the HSF-1 activity, HSP-90 inhibitors/HSP-70 enhancers and chaperone-like molecules. Nevertheless, several of these mechanisms are related to each other as it has been shown that inhibitors targeting HSP-90 can increase the activity of HSF-1, and therefore enhance the transcription of molecules from the Proteostasis network such as HSP-70.

Picture 2. Scheme of current therapeutic approaches. Most of the current therapeutic techniques are focused on inhibition or induction of molecules from the HSP-90 and HSP-70 families. Similar alternatives also have the same output but modulating HSF-1 and HDACs. Another approaches go for direct delivery of HSP-70, chaperone-like molecules and gene therapy.

Knowledge gained through years promoted the development of small molecule inhibitors targeting HSP-90, for example 17-AAG (tanespimycin), to treat several neurodegenerative pathologies and cancers (16). Another inhibitor from the *–mycin* group of molecules (fungi derived antibiotics) is geldanamycin. This antibiotic lead to the development of the group of agents termed as ansamycins. These antibiotics act as competitive inhibitors of ATP, by removing it from the ATP-binding pocket of the N-terminal region in HSP-90 chaperones. Several studies showed a reduction in the oligomerization and aggregation when treated with these type of inhibitors (31). Despite its promising outcome, the use of geldanamycin has been limited because of several reasons: it has poor blood-barrier permeability and poor aqueous solubility, it showed high liver toxicity, and it has been proved that the inhibitory effect is only focused in the early stages of nucleation and protein aggregation (16,31), being useless for the removal or clearance of protein aggregates in later stages. To overcome this issues, several geldanamycin derivatives have been developed, such as the before mentioned tanespimycin, WK-88-1 or the compound 17-DMAG (alvespimycin) (16). Nevertheless, toxicity issues still remain as one of the biggest problems encountered by these type of molecules. Another group of small molecule inhibitors are the ones derived from the compound SNX-2112. These inhibitors promote the degradation of protein aggregates through inhibition of HSP-90 and therefore induction of HSP-70. They present several advantages when compared to ansamycins: the inhibition is selective towards HSP-90, the have good blood-barrier permeability and it has been demonstrated their ability to reduce both oligomeric and fibrilar species of proteins (32). Because of these findings, SNX-2112 derivatives have become a promising molecule for the treatment of neurodegenerative diseases. As mentioned previously in this report, one key factor for the development of protein aggregates is the role of pathological chaperones and promoting agents. Several studies identified these molecules, such as the E4 isoform of ApoE (33), and competitive inhibitors have been developed. These chaperone mimetics bind to the protein aggregate as a normal chaperone would do, and therefore avoid the interaction between the protein aggregate and the pathological chaperone (competitive inhibitor of the chaperone). Another

type of mimetic molecule is the soluble nontoxic oligomer, having higher affinity for the pathological chaperone than the abnormal peptide (competitive inhibitor of the oligomer) (6,33).

On the other hand, several therapeutic strategies have focused in the chaperone induction, and therefore enhance the cell ability to tackle protein misfolding. Two of these enhancers are celastrol and arimoclomol (6,34), both activators of the HSP response via prolongation of the binding to HSF-1 and HSP-70 interaction. Modulation of the HSF-1 activity has received considerable attention as a therapeutic strategy because of the fact that some of these diseases are related to decreased levels of HSP response pathways and chaperone expression. Both celastrol and arimoclomol showed promising results in promoting these mechanisms against several pathologies (34). Furthermore, celastrol has also shown anti-inflammatory and anti-oxidant properties, and besides enhancing HSF-1 activity, it can regulate the binding of HSP-90 to other chaperones (6). Both compounds showed good tolerability and safety in phase I, and are currently ending phase II of their clinical trials. Another related compound is HSF1-A, a small molecule capable of increasing chaperone expression levels by promoting HSF-1 trimerization, its nucleus translocation and phosphorylation. It has been proved to reduce the formation of de-novo protein aggregates in cellular cultures and the animal model of *Drosophila melanogaster* (6). Other studies showed that geranylgeranylacetone is an effective compound to regulate the activity of ERK/p38 MAPK pathways, characteristic in some Alzheimer's disease phenotypes (35). Additionally, it has been proved its role in the induction of HSP-70 via phosphorylation of HSF-1 and stimulation of the protein aggregates clearance. An additional enhancer is valproic acid, a classic anticonvulsive used for epilepsy and bipolar disorders. It has been shown that this compound can inhibit the histone deacetylase (HDAC) activity and act as a HSP-70 inducer, reducing neural apoptosis and oxidative stress (36). Additionally, statins are also becoming an interesting therapeutic approach to target chaperones. Statins are commonly used as inhibitors of HMG-CoA to reduce cholesterol levels in cardiovascular diseases. However, recent studies proved that statins like atorvastatin have anti-apoptotic, anti-oxidative and immunomodulatory effects, as well as having influence over HSP-90 phosphorylation and HSP-27 expression (37). Despite these findings, their role as neuroprotective compounds is very limited, and correlation of long-term treatments with beneficial or detrimental effects still remains inconclusive.

An alternative to increase HSP-70 levels and its activity is by direct administration (38). This study showed the rejuvenation effect in the hippocampus of mice from intranasal administration of HSP-70, proving that HSPs administration can become a promising

approach. One similar alternative to deliver HSPs, is the use cell-penetrating peptides. These molecules are small protein domains capable of transfer compounds through cell membranes and the blood-brain barrier. Delivery of HSP-70 can be accomplish by its fusion to the peptide TAT (trans-activator of transcription, from HIV virus). It has been shown that delivery of HSP-70/TAT complex can reduce toxic protein levels, produce an anti-inflammatory response and induce long-lasting neuroprotective mechanisms (39), showing its potential therapeutic effects. Another one, is the viral vector-mediated upregulation from gene therapy approaches. This technique could be able to restore mutated genes and therefore prevent, reduce or even suppress the pathology. It has been shown that HSP-70 gene transfection using adenoviruses can reduce the apoptosis in some Parkinson's disease phenotypes (40). Other approaches are currently in the first steps of clinical trials (6), but although very promising, gene therapy techniques are still far from becoming a genuine approach.

Finally, an alternative to classic inhibition or enhancing mechanism is the use of molecules with chaperone activity, known as chemical chaperones. These compounds reduce the protein-protein interaction in the protein aggregates, and stabilize the oligomers during refolding. One of these molecules is the disaccharide trehalose. It has been shown that trehalose can bind and stabilize misfolded proteins and avoid its interaction in the aggregate formation. Moreover, it has been proved its inhibition of pro-apoptotic pathways and the clearance of protein aggregates by promoting autophagy (41). Although there are many promising approaches, some issues arise from these techniques as it has been proved that HSPs are strongly upregulated or inhibited in tumor cells and different types of cancers (6,25). Although the regulation of chaperones might not be a trigger for oncogenesis, it may be a key factor in its development.

DISCUSSION AND PROSPECTIVE

Neurodegenerative diseases have been increasing in recent decades, with further rising in the incoming years due to the rapid aging of population. This manifests the importance to study and develop effective therapeutic approaches to tackle them. Currently, the efficiency and development of therapies is hindered due to several factors: one of the main problems relies on the preventive diagnosis, as many of them are diagnosed when the disease is at an advanced state. Biomarkers can be the tool to diagnose the disease in its pre-symptomatic state, as well as monitor its development and finding the appropriate treatment. Different diseases will have their own mechanisms, however, aiming for characteristic biomarkers from the early stages of the disease could lead to more efficient treatments. Although it has been remarkably difficult to find reliable biomarkers, miRNAs are aiming as a promising approach (42). Another issue relies on the reliability of the disease models. This is mainly because

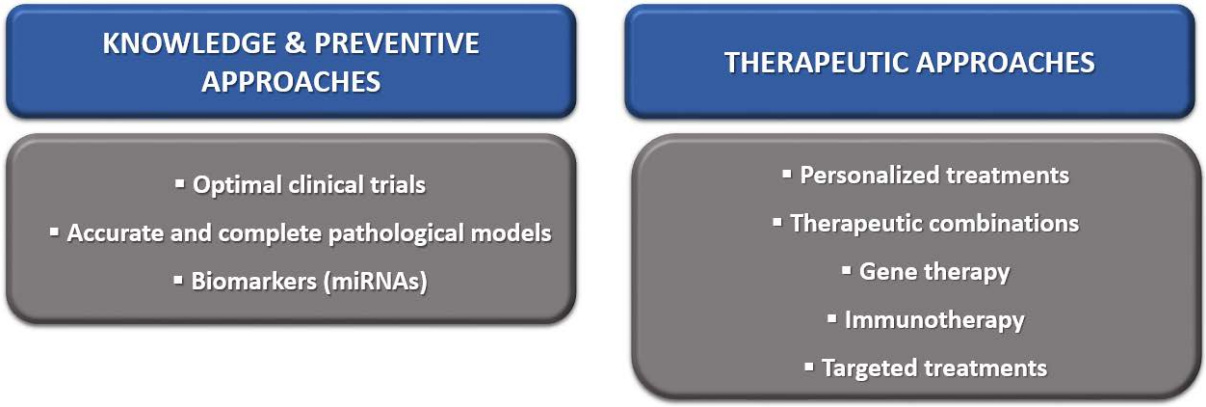
animals models cannot completely mimic all the features of a neurological disease, but rather specific phenotypes or mechanisms (27,35,37). Therefore, by lacking the complete model, the development of a therapy as well as characterising the underlying mechanisms is based in a lack of knowledge. One more problem is the clinical trials, where the selection of population, timing, endpoints and critical outcomes needs to improve as further mentioned in other studies and reviews (4,16,26).

Advancements in the fields of genomics, metabolomics, epigenetics and proteomics can lead to more personalized and effective treatments, while providing more knowledge about the underlying mechanisms causing the disease. Bioinformatics could help in the analysis of big data sets from experimental techniques. In addition, it can provide assistance for next generation sequencing, the –omics branch and synthetic biology to analyse data and develop virtual systems. Enhancement and inhibition of chaperones, especially those targeting the HSP-70 and HSP-90 families stands as a one of the main therapeutic approaches, showing good results against protein aggregation, misfolding and protein toxicity (19,20,24,27,39,43). The problem of therapies focused on HSP-70 and HSP-90 relies in the physiologic role of these chaperones, as they are involved in the systemic homeostasis. They are found in all type of tissues and control protein folding within highly regulated mechanisms. By non-specific targeting, this balance can be disrupted and therefore lead to the development of other undesired conditions and/or diseases. The solution would be to target the HSP-70 and HSP-90 chaperones involved in the neurodegenerative pathology, and limit their influence to the specific mechanism and/or organ of interest. In addition, many of these pathological mechanisms are not isolated, but are part of a bigger pathway. To overcome this problem, a fusion of treatments can be applied, such as a combination of enhancers of HSP-70 or HSF-1 with the inhibition of HSP-90 or pathological chaperones. Furthermore, they can be combined with treatments that promote the protein aggregation clearance via ubiquitin-proteasome activation or the oligomer sequestration.

There are additional alternatives to the classic induction/inhibition of HSP-70 and HSP-90 by drug delivery: gene therapy, protein engineering and immunotherapy. Initial trials have showed that gene therapy can be a safe and valid technique. Advancements in the viral transfection, with more targeted and efficient delivery, and the identification of new target genes are promoting the development of such techniques (26). Although still far from becoming a solid approach, the potential of gene therapy over HSP-70/HSP-90 is increasing on time. On the other hand, protein engineering could allow to modify mutant HSP-70/HSP-90 proteins in order to repair their mutations, improve their stability, and therefore lower their tendency to become pathological chaperones and promote aggregates. This approach

could be combined with the previous mentioned gene therapy, by replacing the protein encoding genes with engineered ones. The other mentioned alternative is the development of immunotherapy as a supplementary treatment to HSP-70/HSP-90 regulation. Using antibodies that target protein aggregation could promote their clearance by the own cell's mechanisms,.

In conclusion, many therapeutic approaches targeting the HSP-70 and HSP-90 families have been and are been developed to tackle neurodegenerative diseases. Some of them fail as they encounter no further benefits or present undesired side effects. Others continue further in their clinical trials, expecting to be the first step to the definitive treatment. As presented here, the delivery of chaperone regulators targeting HSP-70 and HSP-90 have become a promising and valid approach, with several molecules already in the final stages of the clinical trials. Nevertheless, different alternatives arise from the development of new and more efficient techniques (*Picture 3*). The combination of therapeutic improvements and a better understanding of the role of HSP-70 and HSP-90 in the development of the disease puts the final goal one step closer.



Picture 3. Scheme of prospective in neurodegenerative diseases. Preventive approaches rely on the development of more accurate pathological models, improving the understanding of the mechanisms, and develop better biomarkers for early detection. Therapeutic approaches will need to focus on personalized combination of therapies, targeting disease related HSP-70 and HSP-90 proteins, and the help of tools such as genome engineering and immunology.

REFERENCES

1. EU Joint Programme / Neurodegenerative Disease Research. Why choose neurodegenerative diseases? http://www.neurodegenerationresearch.eu/about/why/. Published 2018. Accessed May 19, 2018.
2. Arosio P, Vendruscolo M, Dobson CM, Knowles TPJ. Chemical kinetics for drug discovery to combat protein aggregation diseases. *Trends Pharmacol Sci*. 2014;35(3):127-135. doi:10.1016/j.tips.2013.12.005
3. Soto C. Unfolding the role of protein misfolding in neurodegenerative diseases. *Nat Rev Neurosci*. 2003;4(1):49-60. doi:10.1038/nrn1007
4. Lansbury PT, Lashuel HA. A century-old debate on protein aggregation and neurodegeneration enters the clinic. *Nature*. 2006;443(7113):774-779. doi:10.1038/nature05290
5. Aguzzi A, O'Connor T. Protein aggregation diseases: Pathogenicity and therapeutic perspectives. *Nat Rev Drug Discov*. 2010;9(3):237-248. doi:10.1038/nrd3050
6. Bartolini M, Andrisano V. Strategies for the inhibition of protein aggregation in human diseases. *ChemBioChem*. 2010;11(8):1018-1035. doi:10.1002/cbic.200900666
7. Cohen SIA, Vendruscolo M, Dobson CM, Knowles TPJ. From macroscopic measurements to microscopic mechanisms of protein aggregation. *J Mol Biol*. 2012;421(2-3):160-171. doi:10.1016/j.jmb.2012.02.031
8. Kampinga HH, Bergink S. Heat shock proteins as potential targets for protective strategies in neurodegeneration. *Lancet Neurol*. 2016;15(7):748-759. doi:10.1016/S1474-4422(16)00099-5
9. Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: Progress and problems on the road to therapeutics. *Science (80-)*. 2002;297(5580):353-356. doi:10.1126/science.1072994
10. Campioni S, Mannini B, Zampagni M, et al. A causative link between the structure of aberrant protein oligomers and their toxicity. *Nat Chem Biol*. 2010;6(2):140-147. doi:10.1038/nchembio.283
11. Gazit E. Mechanisms of amyloid fibril self-assembly and inhibition: Model short peptides as a key research tool. *FEBS J*. 2005;272(23):5971-5978. doi:10.1111/j.1742-4658.2005.05022.x
12. Victoria GS, Zurzolo C. The spread of prion-like proteins by lysosomes and tunneling nanotubes: Implications for neurodegenerative diseases. *J Cell Biol*. 2017;216(9):2633-2644. doi:10.1083/jcb.201701047
13. Yvette G. NIH Public Access. *Nat Rev Mol Cell Biol*. 2010;11(4):301-307. doi:10.1038/nrm2873.Prion-like
14. Kundra R, Ciryam P, Morimoto RI, Dobson CM, Vendruscolo M. Protein homeostasis of a metastable subproteome associated with Alzheimer's disease. *Proc Natl Acad Sci*. 2017;114(28):E5703-E5711. doi:10.1073/pnas.1618417114
15. Walter P, Ron D. The unfolded protein response: From stress pathway to homeostatic regulation. *Science (80-)*. 2011;334(6059):1081-1086. doi:10.1126/science.1209038
16. Ebrahimi-Fakhari D. WL_ MP. Molecular Chaperones in Parkinson's Disease – Present and Future. *J Park Dis*. 2011;4(1):299-320. doi:10.1016/j.surg.2006.10.010.Use
17. Collins GA, Goldberg AL. The Logic of the 26S Proteasome. *Cell*. 2017;169(5):792-806. doi:10.1016/j.cell.2017.04.023
18. Kakkar V, Meister-Broekema M, Minoia M, Carra S, Kampinga HH. Barcoding heat shock proteins to human diseases: looking beyond the heat shock response. *Dis Model Mech*. 2014;7(4):421-

434. doi:10.1242/dmm.014563

19. Mannini B, Cascella R, Zampagni M, et al. Molecular mechanisms used by chaperones to reduce the toxicity of aberrant protein oligomers. *Proc Natl Acad Sci*. 2012;109(31):12479-12484. doi:10.1073/pnas.1117799109
20. Cohen SIA, Arosio P, Presto J, et al. Europe PMC Funders Group The molecular chaperone Brichos breaks the catalytic cycle that generates toxic A β oligomers. 2015;22(3):207-213. doi:10.1038/nsmb.2971.The
21. Xu L-Q, Wu S, Buell AK, et al. Influence of specific HSP70 domains on fibril formation of the yeast prion protein Ure2. *Philos Trans R Soc B Biol Sci*. 2013;368(1617):20110410-20110410. doi:10.1098/rstb.2011.0410
22. Li J, Labbadia J, Morimoto RI. Rethinking HSF1 in Stress, Development, and Organismal Health. *Trends Cell Biol*. 2017;27(12):895-905. doi:10.1016/j.tcb.2017.08.002
23. Miyata Y, Iii JK, Kiray J, Dickey CA, Jason E. NIH Public Access. *Futur Med Chem*. 2012;3(12):1523-1537. doi:10.4155/fmc.11.88.Molecular
24. Bertelsen EB, Chang L, Gestwicki JE, Zuiderweg ERP. Solution conformation of wild-type E. coli Hsp70 (DnaK) chaperone complexed with ADP and substrate. *Proc Natl Acad Sci*. 2009;106(21):8471-8476. doi:10.1073/pnas.0903503106
25. Sõti C, Nagy E, Giricz Z, Vígh L, Csermely P, Ferdinandy P. Heat shock proteins as emerging therapeutic targets. *Br J Pharmacol*. 2005;146(6):769-780. doi:10.1038/sj.bjp.0706396
26. O'Connor DM, Boulis NM. Gene therapy for neurodegenerative diseases. *Trends Mol Med*. 2015;21(8):504-512. doi:10.1016/j.molmed.2015.06.001
27. Zhang H, Xu LQ, Perrett S. Studying the effects of chaperones on amyloid fibril formation. *Methods*. 2011;53(3):285-294. doi:10.1016/j.ymeth.2010.11.009
28. Kumita JR, Poon S, Caddy GL, et al. The Extracellular Chaperone Clusterin Potently Inhibits Human Lysozyme Amyloid Formation by Interacting with Prefibrillar Species. *J Mol Biol*. 2007;369(1):157-167. doi:10.1016/j.jmb.2007.02.095
29. Cabaleiro-Lago C, Quinlan-Pluck F, Lynch I, et al. Inhibition of amyloid ?? protein fibrillation by polymeric nanoparticles. *J Am Chem Soc*. 2008;130(46):15437-15443. doi:10.1021/ja8041806
30. Bodner RA, Outeiro TF, Altmann S, et al. Pharmacological promotion of inclusion formation: A therapeutic approach for Huntington's and Parkinson's diseases. *Proc Natl Acad Sci*. 2006;103(11):4246-4251. doi:10.1073/pnas.0511256103
31. McLean PJ, Klucken J, Shin Y, Hyman BT. Geldanamycin induces Hsp70 and prevents α-synuclein aggregation and toxicity in vitro. *Biochem Biophys Res Commun*. 2004;321(3):665-669. doi:10.1016/j.bbrc.2004.07.021
32. Okawa Y, Hideshima T, Steed P, et al. SNX-2112, a selective Hsp90 inhibitor, potently inhibits tumor cell growth, angiogenesis, and osteoclastogenesis in multiple myeloma and other hematologic tumors by abrogating signaling via Akt and ERK. *Blood*. 2009;113(4):846-855. doi:10.1182/blood-2008-04-151928
33. Huang Y.W.A, Zhou B. WM and TCS. ApoE2, ApoE3 and ApoE4 Differentially Stimulate APP Transcription and Aβ Secretion. *Cell*. 2017;168(3):427-441. doi:10.1016/j.coviro.2015.09.001.Human
34. Deane CAS, Brown IR. Induction of heat shock proteins in differentiated human neuronal cells following co-application of celastrol and arimoclomol. *Cell Stress Chaperones*. 2016;21(5):837-848. doi:10.1007/s12192-016-0708-2

35. Sun Y, Zhang JR, Chen S. Suppression of alzheimer's disease-related phenotypes by the heat shock protein 70 inducer, geranylgeranylacetone, in APP/PS1 transgenic mice via the ERK/p38 MAPK signaling pathway. *Exp Ther Med*. 2017;14(6):5267-5274. doi:10.3892/etm.2017.5253

36. Ying GY, Jing CH, Li JR, et al. Neuroprotective effects of valproic acid on blood-brain barrier disruption and apoptosis-related early brain injury in rats subjected to subarachnoid hemorrhage are modulated by heat shock protein 70/matrix metalloproteinases and heat shock protein 70/AKT. *Neurosurgery*. 2016;79(2):286-295. doi:10.1227/NEU.0000000000001264

37. He X, Yang J, Li L, et al. Atorvastatin protects against contrast-induced nephropathy via anti-apoptosis by the upregulation of Hsp27 in vivo and in vitro. *Mol Med Rep*. 2017;15(4):1963-1972. doi:10.3892/mmr.2017.6251

38. Evgen'ev MB, Krasnov GS, Nesterova I V., et al. Molecular mechanisms underlying neuroprotective effect of intranasal administration of human Hsp70 in mouse model of Alzheimer's disease. *J Alzheimer's Dis*. 2017;59(4):1415-1426. doi:10.3233/JAD-170398

39. Doeppner TR, Kaltwasser B, Fengyan J, Hermann DM, Bähr M. TAT-Hsp70 induces neuroprotection against stroke via anti-inflammatory actions providing appropriate cellular microenvironment for transplantation of neural precursor cells. *J Cereb Blood Flow Metab*. 2013;33(11):1778-1788. doi:10.1038/jcbfm.2013.126

40. Dong Z, Wolfer DP, Lipp HP, Büeler H. Hsp70 gene transfer by adeno-associated virusi inhibits MPTP-induced nigrostriatal degeneration in the mouse model of Parkinson disease. *Mol Ther*. 2005;11(1):80-88. doi:10.1016/j.ymthe.2004.09.007

41. Michele KH, Amirhossein C. Autophagy induction by trehalose : Molecular mechanisms and therapeutic impacts. 2018;8(December 2017):1-20. doi:10.1002/jcp.26583

42. Danborg PB, Simonsen AH, Waldemar G, Heegaard NHH. The potential of microRNAs as biofluid markers of neurodegenerative diseases-a systematic review. *Biomarkers*. 2014;19(4):259-268. doi:10.3109/1354750X.2014.904001

43. Narayan P, Orte A, Clarke RW, et al. The extracellular chaperone clusterin sequesters oligomeric forms of the amyloid-β 1-40 peptide. *Nat Struct Mol Biol*. 2012;19(1):79-84. doi:10.1038/nsmb.2191