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HSP- 70 Mediated Nervous System Enhancement by ETAS

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ABSTRACT

Neurodegenerative disease in the CNS is usually a product of increased oxidative stress in the brain. In this study we tested the ability of an asparagus supplement ETAS to help reduce oxidative stress in the normal brains of Balb C mice. Oxidative stress pathways (Heat shock proteins) are usually cumulative in the damage they cause when disrupted. We treated normal Balb C mice with ETAS and had control groups with no ETAS supplementation in their regular diet. We then sacrificed the mice and conducted microarray studies to compare oxidative stress pathway genes. We also characterized the effects of regular oxidative cells in a mammalian cell model treated with alcohol to understand how oxidative stress impairs the functions of a normal neuronal cell. Results from both in vivo and in vitro experiments will be described in this study.

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LIST OF ABBREVIATIONS

ANOVA - One Way Analysis of Variance

APP -Amyloid Precursor Protein

CNS – Central Nervous System

ERK - Extracellular Signal Regulated Kinase

ETAS - Asparagus Officinalis, standardized stem extract

GSH - Glutathione

HSP – Heat Shock Protein

JDP – J Domain Proteins

JNK – C-Jun NH2 Terminal Kinase

LGMD1 – Limb Girdle Muscular Dystrophy

MAPT – Microtubule Associated Protein

NEF's – Nucleotide Exchange Families

NFT - Neurofibrillary tangles

ROS – Reactive Oxygen Species

TTBK Tau Tubulin Kinase

WT - Wild Type

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CHAPTER 1

INTRODUCTION

Neurodegeneration is characterized as the loss of function of neurons in the brain. In neurodegenerative diseases such as Alzheimer's, expression of amyloid precursor protein and hyper phosphorylation of tau protein promotes neuronal dysfunction and cell death. According to the amyloid cascade theory, the accumulation of amyloid β-peptide (Aβ), in the brain is the primary cause of Alzheimer disease (Ricciarelli et al, 2017). Progressive neuronal loss in the brain, biochemical impairment, can also occur as reactive oxygen species, ROS, are produced via oxidative phosphorylation in the mitochondria. Under normal conditions, the harmful effects of ROS are neutralized by antioxidant systems but, when ROS production exceeds the capacity of the antioxidant response system, protein oxidation occurs causing oxidative damage, cellular degeneration, and functional decline at synapses preventing cell to cell communications (Salim et al, 2017).

Tau protein is a microtubule associated protein that functions within neurons of the central nervous system. These proteins play a role in cell signaling, synaptic plasticity and, genomic stability. In humans, TAU can undergo post-translational modifications such as hyperphosphorylation and self-aggregation transforming into insoluble filaments. Tauopathies, neurodegenerative diseases characterized by misfolding of tau protein, can be characterized by the abnormal deposition of microtubule- associated protein tau, MAPT, within neurons and glial cells (Michalicova et al, 2020). Additionally, the co-expression of tau tubulin kinases 1 and 2 (TTBK1/2) leads to increased phosphorylation of tau and ultimately neurodegeneration (Taylor et al, 2018).

The amyloid cascade hypothesis is a proposed mechanism to describe the clinical presentation of neurodegenerative disease associated with the accumulation of Aβ plaques in the brain (Ricciarelli et al, 2017). Amyloid precursor protein is a single- pass transmembrane protein with 8 potential isoforms due to alternative splicing. Mutations at the methionine amino acid is enough to eliminate the ability of Aβ peptides to generate reactive oxidative species. In vivo studies suggest that mice that over express human APPs develop aggregations and consequently express neuronal injury including synaptic dysfunction and loss of synaptic terminals. Soluble Aβ had been shown to control the cleavage and phosphorylation of tau, ultimately both of with play a role in the formation of neurofibrillary tangles (NFT), (O'Brian et al, 2011).

The central nervous system is composed of the brain and spinal cord. Its capability to properly function is dependent upon its ability to maintain ionic, energetic and redox homeostasis. It has now been demonstrated that proteins controlling ATP generation, mitochondrial stability and the redox environment are associated with neurological disorders (Palubinsky et al, 2012). Glutathione (GSH) is arguably one of the most important cellular antioxidants within the central nervous system and is responsible for reducing ROS and maintaining the cellular redox potential defensively against oxidative stress. Internal conditions where there is excessive activation of the glutamate receptors promote a cascade of neurotoxicity via cationic influx, mitochondrial dysfunction, energetic and oxidative stress, and over production of reactive oxidative species (Armanda-Moreira et al, 2020).

Mitochondria are one of the primary contributors to glutamate neurotoxicity. As stated previously, excessive activation of glutamate receptors causes an increased concentration of

calcium ions into neurons. In order to counteract the cationic influx, ATP- dependent ion pumps are activated draining ATP stores and producing neurons with a low energetic state. Ultimately, the excessive calcium uptake can lead to depolarization of the mitochondrial membrane consequently impairing ATP production and antioxidant mitochondrial functions. As mitochondria fail calcium cannot make it to endoplasmic reticulum leading to the production of misfolded or damages proteins and kinases (Palubinsky et al, 2012).

Mammalian heat shock proteins are evolutionary conserved proteins that act as molecular chaperones for other proteins. They are instrumentals for cell to cell signaling and protein traffic. Under normal cell conditions HSP70 will functions as an ATP- dependent molecular chaperone that assists with the folding of newly synthesized proteins, polypeptide complexes, and the transport of protein across cellular membranes (Brunet et al, 2007). Within mitochondria HSP70 blocks the apoptotic pathway at the pre-mitochondrial, mitochondrial, and post-mitochondrial levels (Lanneau et al, 2007).

ETAS, Asparagus officinalis, is a standardized extract produced by Amino Up Co., LTD. It is linked to the inhibition of the apoptosis cascade by heightening the neuronal stress response system via heat shock proteins (Peng et al, 2021).

CHAPTER 2

LITERATURE REVIEW

Asparagus officinalis extract (ETAS®50) is a plant extract that has been known to increase neuroprotective effects and mitigate cognitive impairment via the enhancement of HSP70 expression. Previous studies have shown that ETAS®50 significantly increases HSP70 gene expression in the hippocampus of APP- overexpressing mice when compared to the "wild type", saline treated. Additionally, ETAS®50 significantly decreases soluble and insoluble β-amyloid and tau protein in APP mice when compared to the saline treated group. Previous data also indicates that ETAS®50 reduces caspase-3, decreasing apoptosis at the post mitochondrial level (Peng et al, 2021).

As previously stated, proteins controlling ATP generation, mitochondrial stability and the redox environment are associated with neurological disorders (Palubinsky et al, 2012). HSP70 interacts with mitochondria at the pre-mitochondrial, mitochondrial, and post-mitochondrial levels to regulate redox and ionic homeostasis via protein activation (Lanneau et al, 2007). At the pre-mitochondrial level, HSP70 primarily affects three different cell signaling pathways to induce a neuroprotective effect. These pathways include the mitogen-activated protein kinases, MAPK, extracellular signal-regulated kinase, ERK, and c-Jun NH_2-terminal kinase, JNK and p38 MAPK. The loss in regulation of proteins in MAPK signaling pathways is associated with various neurodegenerative diseases (Eun et al, 2010) In this study we analyze MAPK proteins MAP3K6, MAP3K10, MDK and MLK1. The activation of these various MAPKS is linked to increase in protective effects of HSP70 (Hao et al, 2018; Yu et al, 2010; Miova et al,2015; Yu et al, 2015; Qi et al, 2015; Yu et al, 2013; Fan et al, 2021; Zhang et al, 2018). ERK is a type of MAPK pathway that play a role in neuronal cell death via potassium deprivation (Eun et al,

2010). GAREM1 and KIF26A are proteins that function at the pre mitochondrial level to suppress the cell signaling pathway (Taniguchi et al,2013; Eun et al, 2010; Cheung et al, 2004; Zhou et al, 2009; Mahato et al, 2020; Xu et al, 2006). SH3RF1 is a protein associated with the JNKs cell signaling pathway that plays a role in induction of apoptosis (Xu et al, 2003; Wakatsuki et al, 2022). Lastly, ZNRF1 is activated by p38 MAPK to promote neuronal cell death and neurite degeneration (Stankiewicz et al, 2005).

At the mitochondrial level, HSP70 inhibits Bax translocation and insertion into the outer mitochondrial membrane. This inhibition prevents mitochondrial membrane permeabilization and release of proapoptotic factors cytochrome c and AIF (Lanneau et al, 2007; Stankiewicz et al, 2005). Proteins of relevance at the mitochondrial level include NAV1, ATP10A, and ATP11A. Nav1 is a voltage gated sodium channel that can cause functional change in mitochondria in stressful physiological conditions such as a disrupted ionic balance (Wang et al,2017; Perez-Hernandez et al, 2021; Lezi et al, 2012;Sheng et al, 2012; Johri et al, 2012).

Lastly, at the post- mitochondrial level, HSP70 binds directly to Apaf-1 preventing the recruitment of procaspase-9- to the apoptosome (Lanneau et al, 2007). TRP53BP2 encodes the gene responsible for induction of apoptosis via the 53BP2 proteins and activation of caspase -9 (Kobayashi et al, 2005).

Heat shock proteins are cellular proteins that are highly conserved in evolution. They are ATP-dependent chaperones involved in a multitude of protein folding processes. HSPs have the ability to interact with almost all proteins in their misfolded, unfolded, or aggregated states. HSPs do not interact with proteins in the adequately folded conformation. Essential to the chaperone cycle of HSP70 is the nucleotide-controlled switch between its low and high infinity states.

In the low-affinity state, ATP is bound, meaning association of peptides to the substrate-binding domain and peptide disassociation from the substrate binding domain occur at a significantly high rate ultimately resulting in a low affinity for polypeptides. The high-affinity state occurs following ATP hydrolysis by the non-binding domain. In this state peptide association and dissociation rates decrease by several magnitudes resulting in an increase in affinity for substrate peptides by up to 400-fold. Essentially, ATP hydrolysis is a crucial component of the chaperone activity of HSP70.

HSPs usually do not function alone but with co-chaperones. Arguably, one of the most essential co-chaperones functions within the J-domain proteins (JDPs) and subsequently nucleotide exchange families, NEFs). These JDFs either bind to HSP70 substrates themselves or are located in a cellular location where relevant substrates frequently appear. The mechanism of JDPs can be described as a coupling transfer of polypeptides to HSP70s via stimulation of their ATPase activity (Mayer et al, 2013)

Hoshino et al completed studies in 2011 to identify the relationship between HSP70 beta-amyloid and tau. They reported significant improvement in behavior and cognitive perception following a decreased burden of amyloid plaques in Alzheimer's disease mouse models expressing a high-level HSP70 compared to the control strain. Additionally, the presence of HSP70 increases the degradation of beta-amyloid peptides in microglial cells of the central nervous system. Similarly, Evans et al described how the presence of HSP70 decreases the number of oligomers via refolding of misfolded proteins. Uniformly, related studies have shown that the overexpression of HSP70 is associated with reduced tau protein gene concentration by binding to tau protein and preventing its aggregation by redirecting through ubiquitin degradation pathways (Repalli et al, 2015)

The structure of HSP70 is composed of three domains. At the N-terminal ATPase domain, the exchange of ATP drives conformational changes in the other two domains; the substrate-binding domain includes a groove that allows for polypeptide interaction and binding; the c-terminal domain, or non-binding domain, is rich in alpha-helical structures that function as a "lid" for the substrate-binding domain. In brief, the substrate-binding domain is open when ATP is bound and closed when ADP is bound.

A protein folding machine, HSP70 binds and releases hydrophobic DNA sequences via a regulated ATP-hydrolysis-driven cycle. HSP70 is usually in the ATP-bound state with little to no ATPase activity meaning spontaneous hydrolysis will not occur when substrate interaction is not occurring. HSP70 recognizes and interacts with hydrophobic amino acid sequences within newly synthesized proteins from ribosomes. These proteins can be freely bound and released by the ATPase of ATP70. The ATPase activity of HSP70 is stimulated by the presence of a polypeptide in the peptide-binding domain, resulting in a slow rate of ATP hydrolysis As ATP is hydrolyzed ADP the binding domain of HSP70 closes trapping the polypeptide chain. The polypeptide binding to HSP70 prevents the substrate from further aggregation or misfolding rendering it non-functional. As the protein is synthesized a nucleotide exchange factor stimulates the release of ADP and binding of fresh ATP by opening the binding pocket. Once the protein is released, it is then free to fold correctly on its own or be transferred to another chaperone for further processing.

HSP70 is in the cytoplasm, endoplasmic reticulum, nucleus, mitochondria, and extracellular environment. In relation to neurodegeneration, it associates with APP and tau protein to mitigate the presentation of cognitive decline associated with neurodegenerative conditions. HSP70 functions to correctly fold client proteins, prevent the aggregation of unfolded

or misfolded proteins and exhibit immune-modulatory effects. As a result, within pharmacological testing scientists may focus on the binding of various allosteric sites through its interaction to misfolded tau protein and general modulation of HSP70 expression levels. (Campanella et al, 2018).

HSP40 functions as a co-chaperone to HSP70 through direct substrate binding. Once HSP40 binds to the polypeptide then transferal to HSP70 occurs allowing for subsequent processing. Previous studies have shown that HSP40 specifically binds to misfolded or denatured polypeptide chains. This interaction is heavily influenced by ATPase activity within HSP70. It is theorized that J-domain- HSP70 binding functions to bring the substrate in close proximity to the HSP70 substrate binding site. Following the hydrolysis of ATP, substrate affinity increases and HSP70 can out-compete HSP40 for the substrate binding. In summary, polypeptide release from HSP70 is mediated by the exchange of ADP for ATP. (Lui et al, 2020)

The maintenance of homeostasis especially protein homeostasis is vitally important in specialized cells such as neurons. DNAJ proteins have been demonstrated to play a direct role in protecting against neurodegenerative conditions caused by misfolded or aggregated proteins. DNAJ proteins or HSP40 proteins are chaperones that regulate HSP70 activity through stimulation of ATP hydrolysis. These proteins can be divided into three classes depending on their domain composition. Class I, DNAJA, contains the standard domain structure of an N terminal J domain followed by a glycine and phenylalanine rich region, a zinc finger motif and a C terminal client binding domain. Class II, DNAJB proteins, only contain the N terminal J domain region and glycine/ phenylalanine regions. Lastly, class III, DNAJC, only has the J domain.

DNAJB6 is a highly expressed protein that can primarily be found in the brain. The alternative spicing of DNAJB6 produces two isoforms a nuclear isoform and a cell stress responsive isoform. It has been previously demonstrated that mutations in the DNAJB6 domain led to neurodegeneration specifically, limb-girdle muscular dystrophy. LGMD1 is a neurological condition that causes progressive muscle weakness and degeneration.

DNAJB1 has been shown to enhance the neuroprotective effects of HSP70 in vitro by reducing alpha-beta aggregation by targeting smaller oligomers. Additionally, DNAJA1 has been shown to regulate mutant tau fate in the presence of HSP70 by stabilizing and preventing degradation (Zarouchlioti et al, 2018)

The STRING database is a database of functional associations between proteins using the fact that functionally associated proteins are generally encoded by genes that share similar selection pressures. As a result, those genes are maintained and regulated together in a way that the encoded can interact at the same place and time in the cell. The STRING database is a computerized resource that is used to explore the nature of these associations. The STRING database is composed of a unique scoring framework founded on various benchmarks of different pre-determined associations against the reference set. These associations are integrated into a single confidence score per prediction. A representation of the network of the proposed protein interactions creates a view of the predicted functional linkage. The STRING database predicts functional interactions at an expected level of accuracy of 80% or higher for most genes (Von Mering et al, 2003).

The foundation of the STRING analysis is based on functional associations. Functional associations are inks between two proteins that contribute to a shared biological function. In order for proteins to be considered to share a function, they do not need to interact physically. If

the two proteins have functional overlap within the same cell then the overlap should fall under a functional pathway in order to be considered relevant. It is important to note that under these conditions, even proteins with a negative functional relationship will be identified using the STRING analysis. (Szklarczky et al, 2019)

CHAPTER 3

MATERIALS AND METHODS

3.1 ETAS®50. The manufacturing process of ETAS®50 starts with the asparagus plant Natural active components are extracted using the hot water extraction method. The water is then filtered through a filtration system and concentrated. The concentrate is then spray dried into powder (Amino up, 2021).

3.2 Gene Array Analysis. RNA Extraction and Purification: Gene Expression Quantification Using the Afermetrics Gene Array. RNA extraction and purification was completed by Dr.Sundaresan and Dr.Mann using the following general methodology. Mice were divided into groups of 10 to form 5 experimental groups. Total RNA was extracted from the hippocampus using RNeasy Microarray mini tissue kits. The RNA was then purified so that integrity could be assessed. RNA integrity values below 6 were not used in the acquired data. Next, total RNA was converted into cDNA and amplified using one color labeling kits from Agilent. Finally, the arrays were assesed using BRB Array tools. That data was then normalized and corrected using univariate analysis (Peng et al, 2021).

3.3 String Analysis. Data from the gene array provided by Dr. Sundaresan was input into the STRING database to find predicted functional partners within cell signaling pathways at various cellular levels. Starting with HSP70 as the protein of interest. I used the produced string analysis to filter 309 additional genes identified from the provided gene array. In the produced string analysis-colored nodes represented specific proteins produced by a single protein coding locus. Edges represented protein to protein interactions between functional proteins.

CHAPTER 4

RESULTS

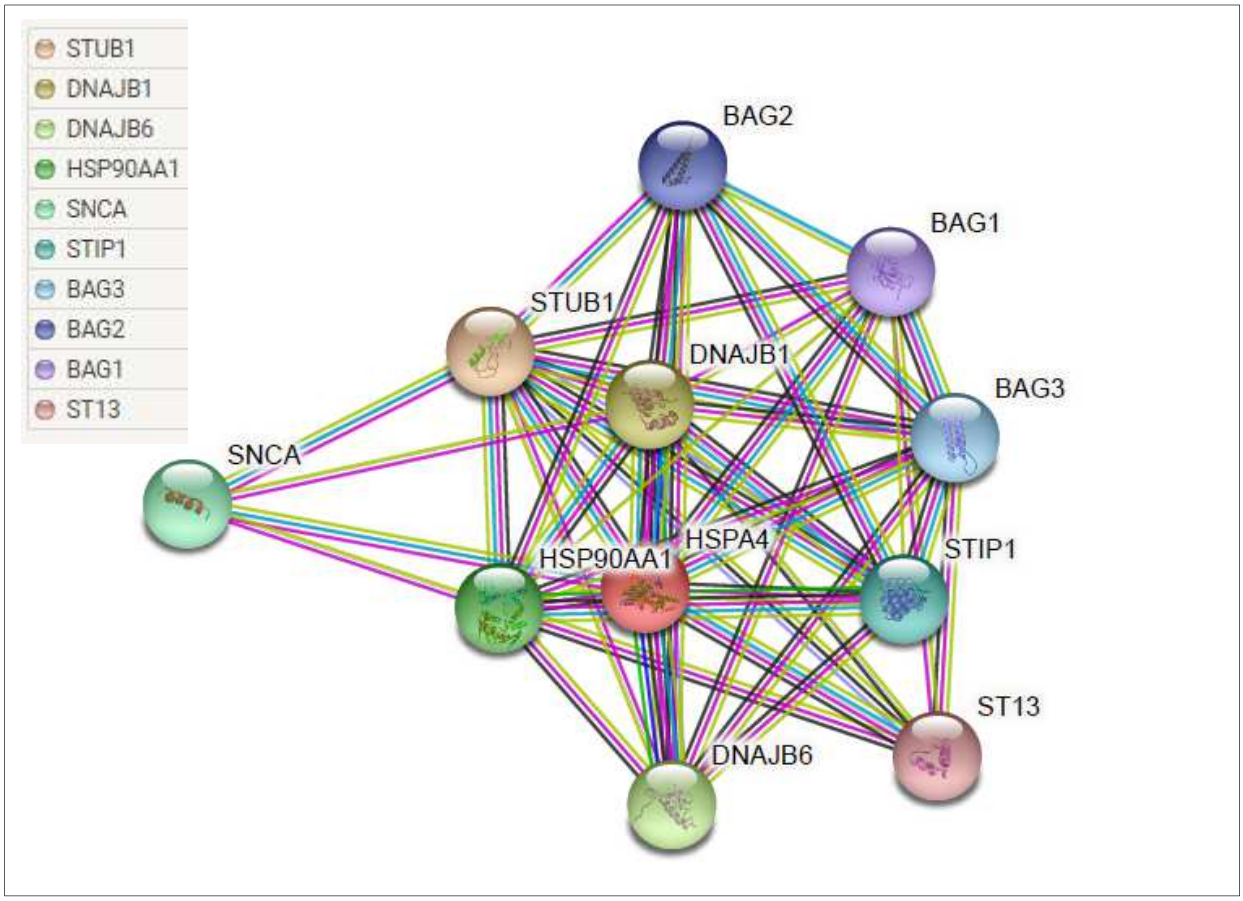


Figure I String Analysis of HSP70. Colored nodes recognize specific proteins produced by a single protein coding locus. Edges represent meaningful protein to protein interactions.

String Analysis: The string analysis of heat shock protein 70 was evaluated using the STRING database. This model is used to predict functional partners between interacting proteins that contribute to a shared function. Figure 1 identifies the predicted functional partners for HSP70. Within the first shell of interactions, DNAJB6, is located. The produced models indicate that HSP70 and DNAJB6, HSP40, interact closely in that they contribute too many shared functions. The above model also highlights other interacting gene families such as BAG and SCNA.

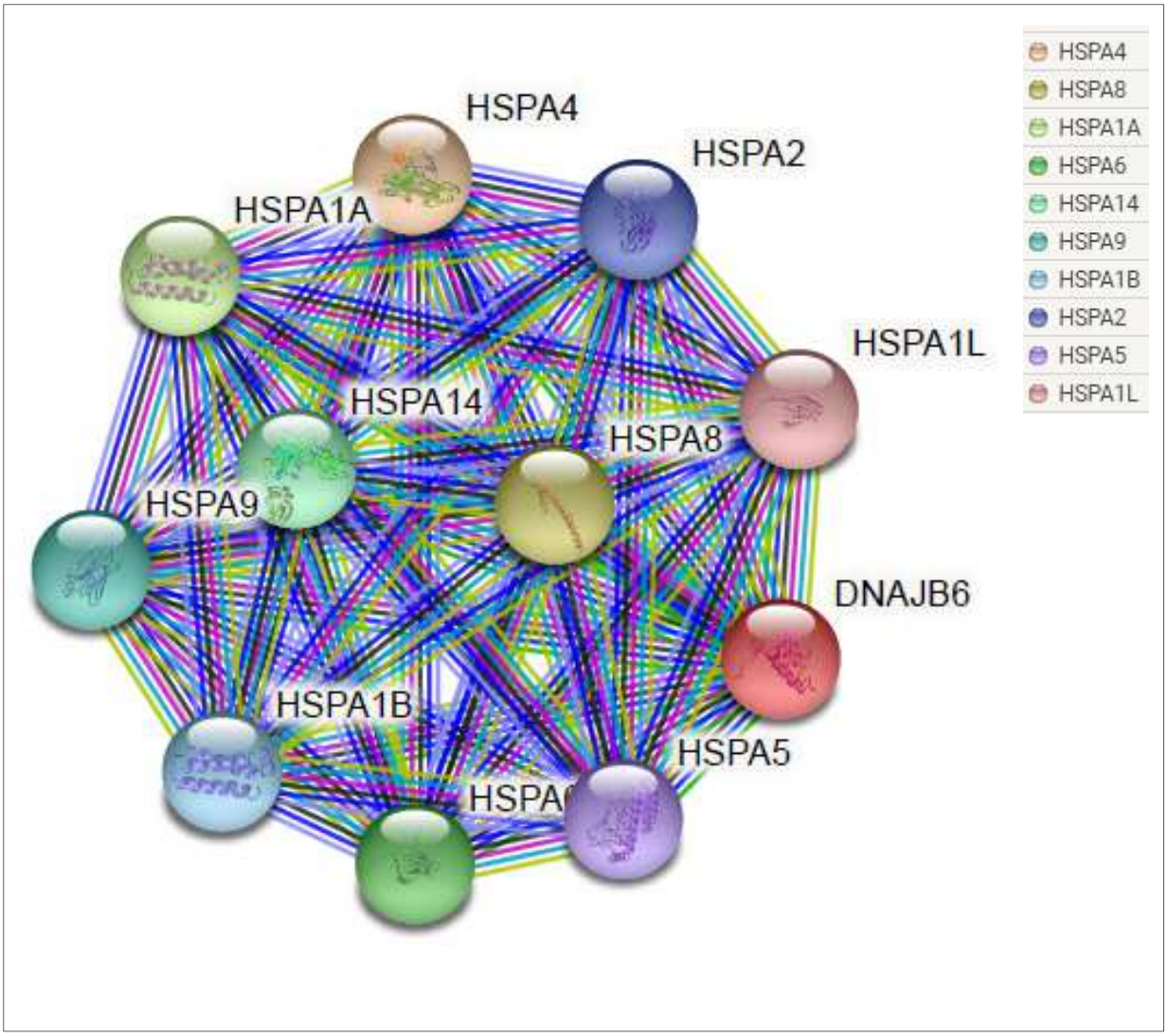


Figure II String Analysis DNAJB6, HSP40. Colored nodes recognize specific proteins produced by a single protein coding locus. Edges represent meaningful protein to protein interactions.

String Analysis: The string analysis of DNAJB6 was evaluated using the STRING database. The predicted model demonstrates a closely interwoven relationship between the gene products of the HSP gene family. This is expected as HSP70 and HSP40 interact to form a complex that exhibits anti-neurodegenerative properties.

Proposed Mechanism of HSP70 and DNAJB6/HSP40

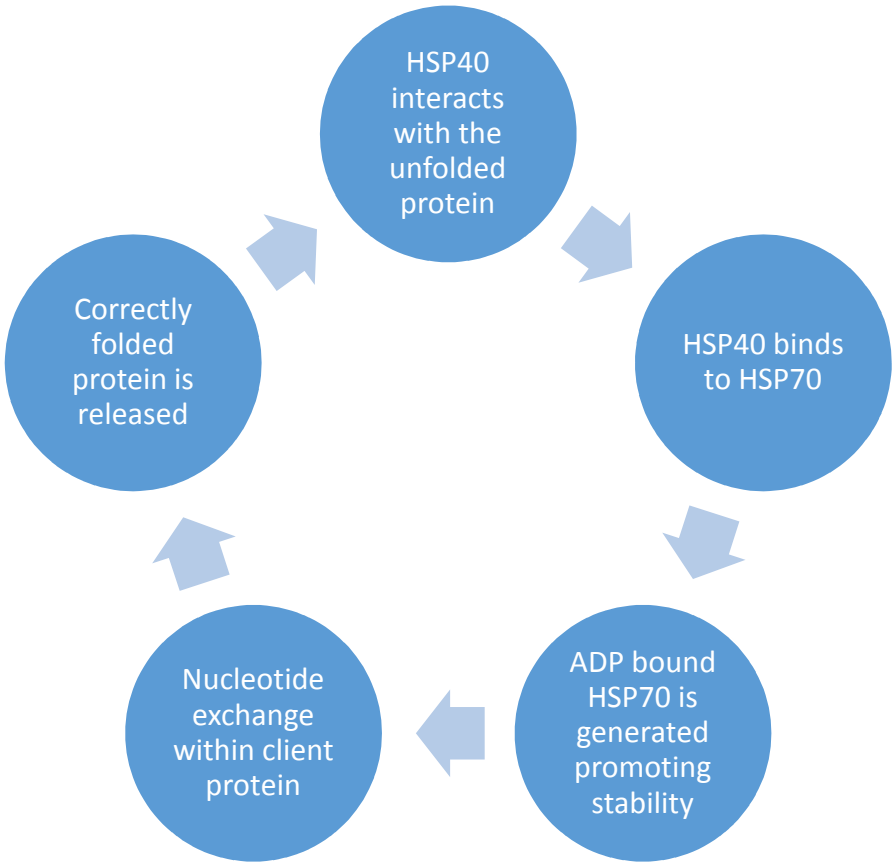
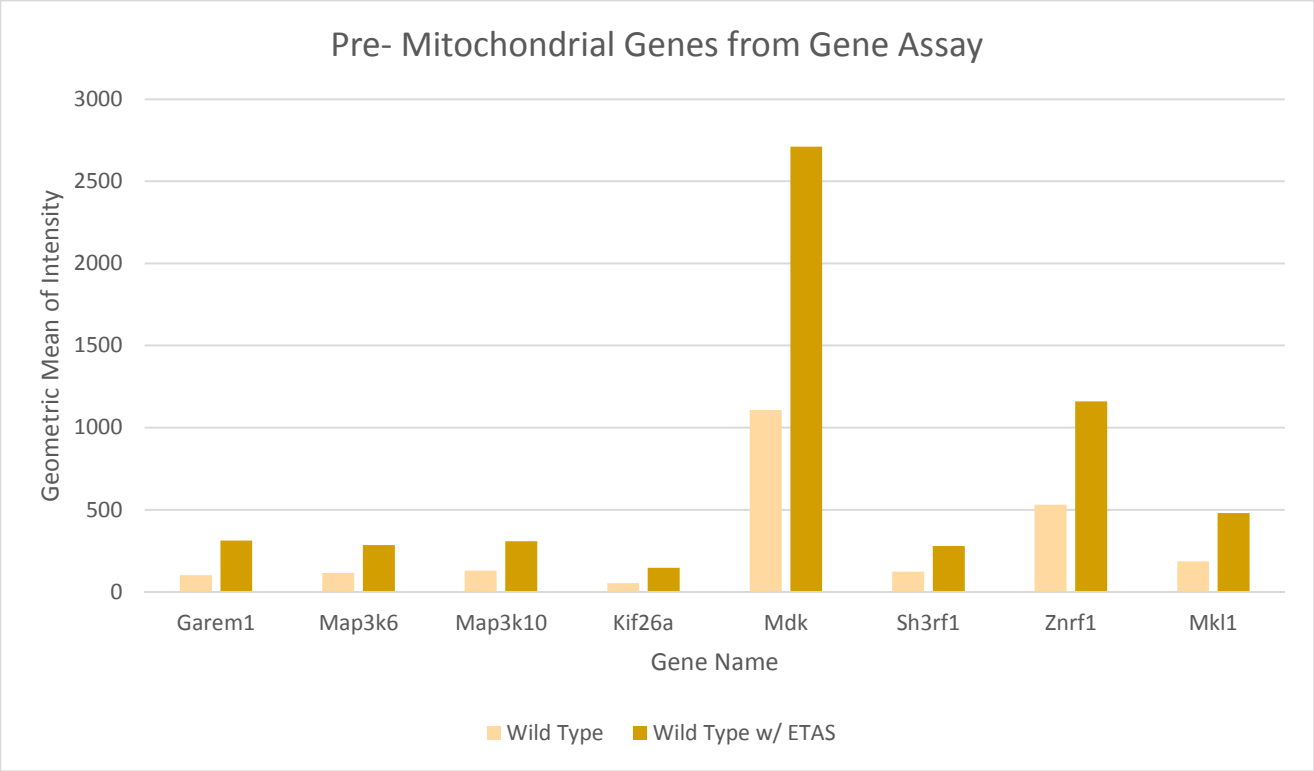


Figure III: Mechanism of chaperone-assisted protein folding by Hsp70-DnaJ/Hsp40 complex.

In the proposed model, DNAJB6, HSP40, interacts with the unfolded protein for delivery to HSP70. HSP40 and HSP70 form a complex that triggers activation of ATPase activity. Activation of the ATPase activity leads to the hydrolysis of ATP to ADP and the release of HSP40 leaving the HSP70/ ADP/ protein complex. Nucleotide exchange occurs allowing for the correctly folded protein to be released and phosphorylation of ADP to ATP. [47].

Relevant Genes at the Pre- Mitochondrial Level



Graph 1: Quantification of pre-mitochondrial gene products in wild type and wild type following exposure to ETAS

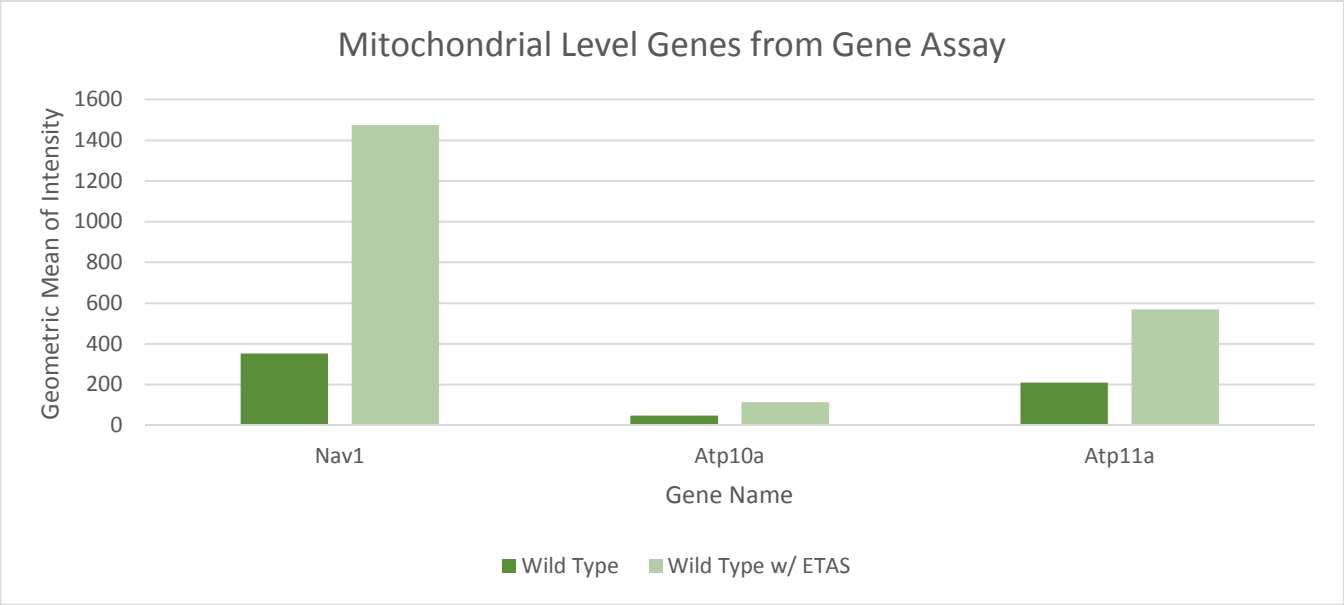
In order to measure the effects of ETAS on neuronal cells, mice were divided into the following groups: wild type and WT with ETAS and, treated with the asparagus supplement, ETAS. Figure one indicates the quantitative change measured in genes known to associate with HSP70 at the pre-mitochondrial level. In the above graph the x-axis corresponds to the gene names and y-axis to geometric mean of intensity/the recorded gene product concentration. The light and dark colored bars represent the wild type and wild type after exposure to etas. The results suggest that the presence of ETAS increases the concentration of gene products that function in a neuroprotective manner.

GENE NAME	WILD TYPE	WILD TYPE W/ ETAS	FOLD CHANGE
GAREM1	100.9	312.16	3.0938
MAP3K6	115.34	284.47	2.4664
MAP3K10	128.22	307.96	2.4018
KIF26A	53.71	145.82	2.7150
MDK	1106.62	2710.73	2.4496
SH3RF1	123.73	278.83	2.2535
ZNRF1	530.12	1160.5	2.1891
MKL1	185.61	480.17	2.5870

Table 1: Relevant Genes at the Pre- Mitochondrial Level

Quantification of gene products were measured in the wild type pre-and post-exposure to ETAS. Those gene products were then analyzed to calculate the fold change of the wild type under the influence of the asparagus supplement. The formula used to calculate fold change was $\frac{Wild\ type\ w\ etas}{wild\ type}$. The fold change is indicative of the quantitative change between the original and subsequent measurement. The results show that in the presence of ETAS, the fold change of gene products that behave in a neuroprotective manner increase significantly with gene products doubling and tripling in the wild type w/ etas compared to the wild type.

Relevant Genes at the Mitochondrial Level



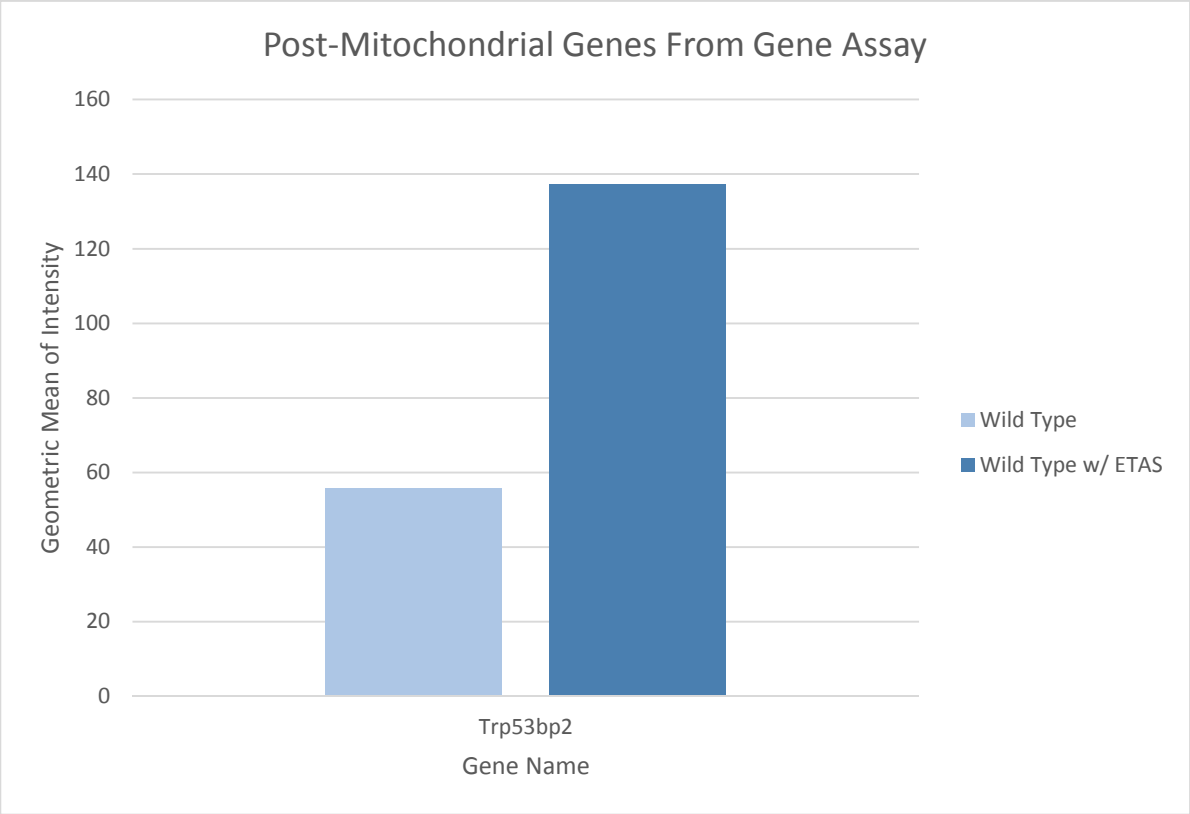
Graph 2: Quantification of mitochondrial level gene products in wild type and wild type following exposure to ETAS

As previously described, the gene products were measured in the wild type before and after exposure to ETAS. Graph two indicates the genes that interact with HSP70 at the mitochondrial level. Following exposure to ETAS, the following mitochondrial gene products increased in concentration: NAV1, ATP10A, and ATP11A. As shown below in table two, the fold change increases by a magnitude of four in NAV1, a protein that function apart of the voltage gated sodium channel and doubles ATPase activity.

GENE NAME	WILD TYPE	WILD TYPE W/ ETAS	FOLD CHANGE
Nav1	353.07	1475.11	4.1780
Atp10a	47.65	113.33	2.7260
Atp11a	209.13	570.09	2.3784

Table 2: Relevant Genes at the Mitochondrial Level

Relevant Genes at the Post- Mitochondrial Level



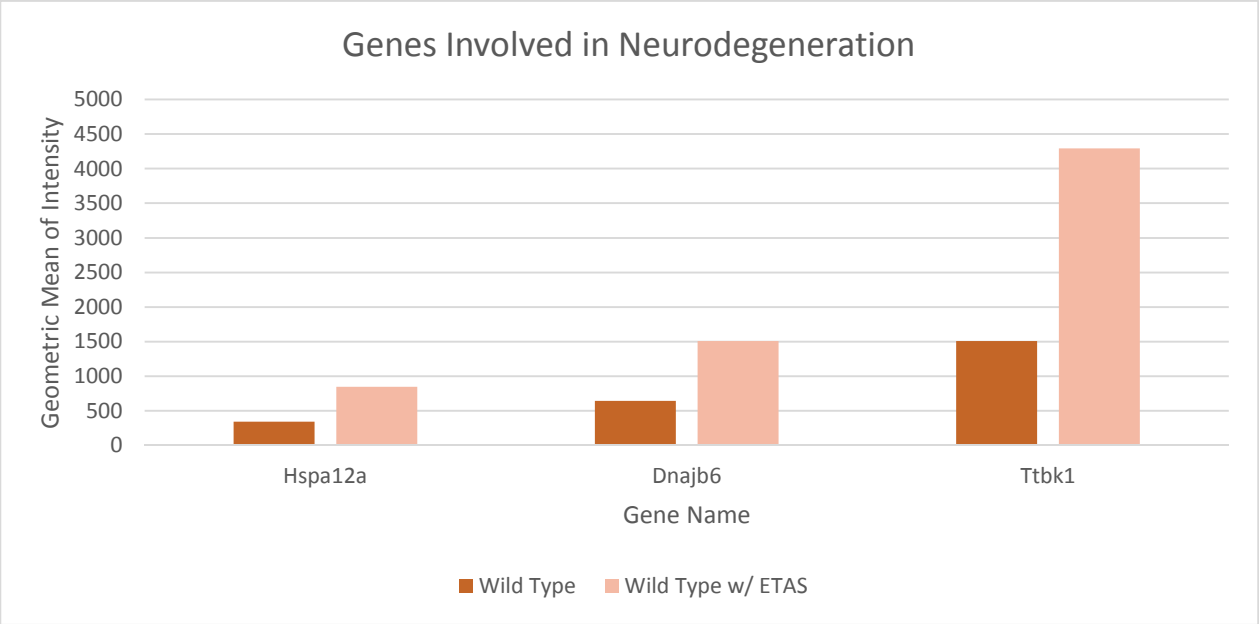
Graph 3: Quantification of post-mitochondrial level gene products in wild type and wild type following exposure to ETAS

Graph 3 summarizes the quantitative change in concentration of gene products that associate with HSP70 at the post mitochondrial level. Using the provided gene Assay I identified a single gene. TRP53BP2 is shown to increase in concentration in the wild type with ETAS compared to the wild type control. As shown in Table 3 the fold change increased at a proportion of 2.46.

GENE NAME	WILD TYPE	WILD TYPE W/ ETAS	FOLD CHANGE
TRP53BP2	55.72	137.23	2.4628

Table 3: Relevant Genes at the Post- Mitochondrial Level

Genes that play a general role in neurodegeneration



Graph 4: Quantification of gene products in wild type and wild type following exposure to ETAS

Graph 4 identifies genes from the provided gene array that play a role in the clinical manifestations of neurodegeneration at a general level including heat shock protein 12, heat shock protein 40, and the tau kinase TTBK1. Following exposure to ETAS, the fold change in the concentration heat shock proteins 12 and 40 doubled by 2.48 and 2.91 respectively as stated in table 4. The fold change of the tau kinase was also shown to double at a rate of 2.84.

Gene Name	Wild Type	Fold Change	Wild Type w/ ETAS
Hspa12a	340.06	2.4807	843.6
Dnajb6	640.34	2.9174	1508.58
Ttbk1	1508.34	2.8480	4295.75

Table 4: Genes that play a General role in Neurodegeneration

CHAPTER 5

DISCUSSION

As described previously, RNA was extracted from wild type mice to quantify the change in gene products following exposure to the asparagus supplement ETAS. Gene expression was quantified using the Agilent Gene Array. The measured gene products were then analyzed in the STRING database using HSP70 as the primary gene of interest to find predicted functional partners and subsequently calculate the fold change of relevant gene products after exposure to ETAS. It has been previously established that ETAS exposure leads to an increase in expression of heat school proteins and decrease in B-amyloid and tau protein in APP over expressed mice [10]. Results at the pre-mitochondrial level support previous studies indicating HSP70 plays a neuroprotective role in the clinical manifestation of neurodegeneration.

Mitochondria have also been established to play a major role in the regulation of neurodegeneration via maintenance of ionic balance within cells. Results from the mitochondrial level presented an increase in ATP activity and active ion pumps. This finding suggests that the presence ETAS acts as a stimulator within neuronal cells. Similarly, the presence of ETAS triggered the activation of TRP53BP2, a caspase 9 activator. The observed changes in gene products at the mitochondrial level and post mitochondrial level indicate some adverse effects neurologically that could potentially be regulated using proper dosage and other clinical mechanisms.

CHAPTER 6

CONCLUSION

In conclusion, in the presence of asparagus supplement, ETAS, an increase in WT mice genes that prevent neurodegeneration and instigate neurodegeneration was observed in genes functioning at the pre-mitochondrial level. Consequently, an increase in gene products that encourage neurodegeneration at the mitochondrial and post mitochondrial level were found to increase as well. Ultimately further investigation will be required to determine if the benefits outweigh the risks in the prevention of neurodegeneration using asparagus supplement, ETAS.

REFERENCES

1. Ricciarelli, R., & Fedele, E. (2017). The Amyloid Cascade Hypothesis in Alzheimer's Disease: It's Time to Change Our Mind. *Current neuropharmacology*, *15*(6), 926–935. https://doi.org/10.2174/1570159X15666170116143743

2. Salim S. Oxidative Stress and the Central Nervous System. J Pharmacol Exp Ther. 2017 Jan;360(1):201-205. doi: 10.1124/jpet.116.237503. Epub 2016 Oct 17. PMID: 27754930; PMCID: PMC5193071.

3. Michalicova, A., Majerova, P., & Kovac, A. (2020). Tau Protein and Its Role in Blood-Brain Barrier Dysfunction. *Frontiers in molecular neuroscience*, *13*, 570045. https://doi.org/10.3389/fnmol.2020.570045

4. Taylor, L. M., McMillan, P. J., Liachko, N. F., Strovas, T. J., Ghetti, B., Bird, T. D., Keene, C. D., & Kraemer, B. C. (2018). Pathological phosphorylation of tau and TDP-43 by TTBK1 and TTBK2 drives neurodegeneration. *Molecular neurodegeneration*, *13*(1), 7. https://doi.org/10.1186/s13024-018-0237-9

5. O'Brien, R. J., & Wong, P. C. (2011). Amyloid precursor protein processing and Alzheimer's disease. *Annual review of neuroscience*, *34*, 185–204. https://doi.org/10.1146/annurev-neuro-061010-113613 Wyttenbach A, Arrigo AP. The Role of Heat Shock Proteins during Neurodegeneration in Alzheimer's,

6. Palubinsky, A. M., Martin, J. A., & McLaughlin, B. (2012). The role of central nervous system development in late-onset neurodegenerative disorders. *Developmental neuroscience*, *34*(2-3), 129–139. https://doi.org/10.1159/000336828

7. Armada-Moreira, A., Gomes, J. I., Pina, C. C., Savchak, O. K., Gonçalves-Ribeiro, J., Rei, N., Pinto, S., Morais, T. P., Martins, R. S., Ribeiro, F. F., Sebastião, A. M., Crunelli, V., & Vaz, S. H. (2020). Going the Extra (Synaptic) Mile: Excitotoxicity as the Road Toward Neurodegenerative Diseases. *Frontiers in cellular neuroscience*, *14*, 90. https://doi.org/10.3389/fncel.2020.00090

8. Brunet, M. & Mirjolet, Celine & Subramaniam, S. & Rérole, A.L. & Thonel, A. & Garrido, Carmen. (2007). Hsp70 and Hsp27 as pharmacological targets in apoptosis modulation for cancer therapy. 10.1007/978-1-4020-6401-2_11.

9. Lanneau, David & Thonel, Aurelie & Maurel, Sebastien & Mirjolet, Celine & Garrido, Carmen. (2007). Apoptosis versus cell differentiation: Role of heat shock proteins HSP90, HSP70 and HSP27. Prion. 1. 53-60. 10.4161/pri.1.1.4059.

10. Peng Z, Bedi S, Mann V, Sundaresan A, Homma K, Gaskey G, Kowada M, Umar S, Kulkarni AD, Eltzschig HK, Doursout MF. Neuroprotective Effects of Asparagus officinalis Stem Extract in Transgenic Mice Overexpressing Amyloid Precursor Protein. J Immunol Res. 2021 May 10; 2021:8121407. Doi: 10.1155/2021/8121407. PMID: 34046506; PMCID: PMC8128539.

11. ATCC. (2022). *SH-SY5Y*. ATCC. Retrieved May 6, 2022, from https://www.atcc.org/products/crl-2266

12. Parkinson's and Huntington's Disease. In: Madame Curie Bioscience Database [Internet]. Austin (TX): Landes Bioscience; 2000-2013. Available from:

https://www.ncbi.nlm.nih.gov/books/NBK6495/?msclkid=7e15bab4b68d11ecaedaaf4264c08398

13. Hardy, J. & Selkoe, D. J. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science 297, 353–356 (2002).

14. Mosser, Richard & Morimoto, Richard. (2004). Mosser DD, Morimoto RI... Molecular chaperones and the stress of oncogenesis. Oncogene 23: 2907-2918. Oncogene. 23. 2907-18. 10.1038/sj.onc.1207529.

15. Venkataraman A, Kalk N, Sewell G, Ritchie CW, Lingford-Hughes A. Alcohol and Alzheimer's Disease-Does Alcohol Dependence Contribute to Beta-Amyloid Deposition, Neuroinflammation and Neurodegeneration in Alzheimer's Disease? Alcohol. 2017 Mar 9; 52(2):151-158. Doi: 10.1093/alcalc/agw092. Erratum in: Alcohol. 2017 Mar 9; 52(2):158. PMID: 27915236.

16. Jeong S. (2017). Molecular and Cellular Basis of Neurodegeneration in Alzheimer's disease. *Molecules and cells*, *40*(9), 613–620. https://doi.org/10.14348/molcells.2017.0096

17. Beckmann RP, Lovett M, Welch WJ. Examining the function and regulation of hsp 70 in cells subjected to metabolic stress. J Cell Biol. 1992 Jun;117(6):1137-50. doi: 10.1083/jcb.117.6.1137. PMID: 1607378; PMCID: PMC2289495.

18. Beere, Helen & Wolf, Beni & Cain, Kelvin & Mosser, Richard & Mahboubi, Artin & Kuwana, Tomomi & Tailor, Pankaj & Morimoto, Richard & Cohen, Gerald & Green, Douglas. (2000). Beere HM, Wolf BB, Cain K, Mosser DD, Mahboubi A, Kuwana T, Tailor P, Morimoto RI, Cohen GM, Green DRHeat-shock protein 70 inhibits apoptosis by

preventing recruitment of procaspase-9 to the Apaf-1 apoptosome. Nat Cell Biol 2:469-475. Nature cell biology. 2. 469-75. 10.1038/35019501.

19. H.J. Jang, J.H. Kwak, E.Y. Cho, Y.M. We, Y.H. Lee, S.C. Kim, D.J. Han,Glutamine Induces Heat-Shock Protein-70 and Glutathione Expression and Attenuates Ischemic Damage in Rat Islets,Transplantation Proceedings,Volume 40, Issue 8,2008,Pages 2581-2584,ISSN 0041.1345,https://doi.org/10.1016/j.transproceed.2008.08.075.(https://www.sciencedirect.com/science/article/pii/S0041134508011536)

20. Meng E, Shevde LA, Samant RS. Emerging roles and underlying molecular mechanisms of DNAJB6 in cancer. Oncotarget. 2016 Aug 16;7(33):53984-53996. doi: 10.18632/oncotarget.9803. PMID: 27276715; PMCID: PMC5288237.

21. Hussein RM, Hashem RM, Rashed LA. Evaluation of the amyloid beta-GFP fusion protein as a model of amyloid beta peptides-mediated aggregation: a study of DNAJB6 chaperone. Front Mol Neurosci. 2015 Jul 27;8:40. doi: 10.3389/fnmol.2015.00040. PMID: 26283911; PMCID: PMC4515555

22. Eun Kyung Kim, Eui-Ju Choi,Pathological roles of MAPK signaling pathways in human diseases, Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease, Volume 1802, Issue 4, 2010, Pages 396-405, ISSN 0925-4439, https://doi.org/10.1016/j.bbadis.2009.12.009. (https://www.sciencedirect.com/science/article/pii/S0925443910000153)

23. Hao, Y., Feng, Y., Li, J., & Gu, X. (2018). Role of MAPKs in HSP70's Protection against Heat Stress-Induced Injury in Rat Small Intestine. *BioMed research international*, *2018*, 1571406. https://doi.org/10.1155/2018/1571406

24. . Yu J., Yin P., Liu F., et al. Effect of heat stress on the porcine small intestine: a morphological and gene expression study. *Comparative Biochemistry and Physiology—A Molecular and Integrative Physiology.* 2010;156(1):119–128. doi: 10.1016/j.cbpa.2010.01.008.

25. Miova B., Dinevska-Kjovkarovska S., Esplugues J. V., Apostolova N. Heat stress induces extended plateau of Hsp70 accumulation—a possible cytoprotection mechanism in hepatic cells. *Journal of Cellular Biochemistry.* 2015;116(10):2365–2374. doi: 10.1002/jcb.25187.

26. Yu J., Jiang Z., Ning L., et al. Protective HSP70 induction by Z-ligustilide against oxygen-glucose deprivation injury via activation of the MAPK pathway but not of HSF1. *Biological & Pharmaceutical Bulletin.* 2015;38(10):1564–1572. doi: 10.1248/bpb.b15-00352.

27. Qi Z., Qi S., Gui L., Shen L., Feng Z. Daphnetin protects oxidative stress-induced neuronal apoptosis via regulation of MAPK signaling and HSP70 expression. *Oncology Letters.* 2016;12(3):1959–1964. doi: 10.3892/ol.2016.4849

28. Yu J., Liu F., Yin P., et al. Involvement of oxidative stress and mitogen-activated protein kinase signaling pathways in heat stress-induced injury in the rat small intestine. *Stress.* 2013;16(1):99–113. doi: 10.3109/10253890.2012.680526.

29. Fan, Y. C., Hsu, K. C., Lin, T. E., Zechner, D., Hsu, S. P., & Tsai, Y. C. (2021). Investigation of Anti-Tumor Effects of an MLK1 Inhibitor in Prostate and Pancreatic Cancers. *Biology*, *10*(8), 742. https://doi.org/10.3390/biology10080742

30. Zhang Z, Qin Z. Characterization of Midkine in tongue sole (Cynoglossus semilaevis)and its role on the germ layer genesis in zebrafish (Danio rerio). Comp Biochem Physiol B Biochem

Mol Biol. 2018 Dec;226:64-72. doi: 10.1016/j.cbpb.2018.08.003. Epub 2018 Aug 13. PMID: 30114527.

31. Taniguchi T, Tanaka S, Ishii A, Watanabe M, Fujitani N, Sugeo A, Gotoh S, Ohta T, Hiyoshi M, Matsuzaki H, Sakai N, Konishi H. A brain-specific Grb2-associated regulator of extracellular signal-regulated kinase (Erk)/mitogen-activated protein kinase (MAPK) (GAREM) subtype, GAREM2, contributes to neurite outgrowth of neuroblastoma cells by regulating Erk signaling. J Biol Chem. 2013 Oct 11;288(41):29934-42. doi: 10.1074/jbc.M113.492520. Epub 2013 Sep 3. PMID: 24003223; PMCID: PMC3795291

32. Eun Kyung Kim, Eui-Ju Choi,Pathological roles of MAPK signaling pathways in human diseases, Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease, Volume 1802, Issue 4, 2010, Pages 396-405, ISSN 0925-4439, https://doi.org/10.1016/j.bbadis.2009.12.009. (https://www.sciencedirect.com/science/article/pii/S0925443910000153)

33. Cheung EC, Slack RS. Emerging role for ERK as a key regulator of neuronal apoptosis. Sci STKE. 2004 Sep 14;2004(251):PE45. doi: 10.1126/stke.2512004pe45. PMID: 15383672.

34. Zhou R, Niwa S, Homma N, Takei Y, Hirokawa N. KIF26A is an unconventional kinesin and regulates GDNF-Ret signaling in enteric neuronal development. Cell. 2009 Nov 13;139(4):802-13. doi: 10.1016/j.cell.2009.10.023. PMID: 19914172.

35. Mahato, A. K., & Sidorova, Y. A. (2020). RET Receptor Tyrosine Kinase: Role in Neurodegeneration, Obesity, and Cancer. *International journal of molecular sciences*, *21*(19), 7108. https://doi.org/10.3390/ijms21197108

36. Xu Z, Greene LA. Activation of the apoptotic JNK pathway through the Rac1-binding scaffold protein POSH. Methods Enzymol. 2006;406:479-89. doi: 10.1016/S0076-6879(06)06036-8. PMID: 16472680.

37. Xu Z, Kukekov NV, Greene LA. POSH acts as a scaffold for a multiprotein complex that mediates JNK activation in apoptosis. EMBO J. 2003 Jan 15;22(2):252-61. doi: 10.1093/emboj/cdg021. PMID: 12514131; PMCID: PMC140096.

38. Wakatsuki S, Takahashi Y, Shibata M, Araki T. Selective phosphorylation of serine 345 on p47-phox serves as a priming signal of ROS-mediated axonal degeneration. Exp Neurol. 2022 Jun;352:114024. doi: 10.1016/j.expneurol.2022.114024. Epub 2022 Feb 23. PMID: 35218706.

39. Stankiewicz AR, Lachapelle G, Foo CP, Radicioni SM, Mosser DD. Hsp70 inhibits heat-induced apoptosis upstream of mitochondria by preventing Bax translocation. J Biol Chem. 2005 Nov 18;280(46):38729-39. doi: 10.1074/jbc.M509497200. Epub 2005 Sep 19. PMID: 16172114.

40. Wang, J., Ou, S. W., & Wang, Y. J. (2017). Distribution and function of voltage-gated sodium channels in the nervous system. *Channels (Austin, Tex.)*, *11*(6), 534–554. https://doi.org/10.1080/19336950.2017.1380758

41. Pérez-Hernández M, Leo-Macias A, Keegan S, Jouni M, Kim JC, Agullo-Pascual E, Vermij S, Zhang M, Liang FX, Burridge P, Fenyo D, Rothenberg E, Delmar M. Structural and Functional Characterization of a $Na_v1.5$-Mitochondrial Couplon. Circ Res. 2021 Feb 5;128(3):419-432. doi: 10.1161/CIRCRESAHA.120.318239. Epub 2020 Dec 21. PMID: 33342222; PMCID: PMC7864872.

42. Lezi, E., & Swerdlow, R. H. (2012). Mitochondria in neurodegeneration. *Advances in experimental medicine and biology*, *942*, 269–286. https://doi.org/10.1007/978-94-007-2869-1_12

43. Sheng, Z. H., & Cai, Q. (2012). Mitochondrial transport in neurons: impact on synaptic homeostasis and neurodegeneration. *Nature reviews. Neuroscience*, *13*(2), 77–93. https://doi.org/10.1038/nrn3156

44. Johri, A., & Beal, M. F. (2012). Mitochondrial dysfunction in neurodegenerative diseases. *The Journal of pharmacology and experimental therapeutics*, *342*(3), 619–630. https://doi.org/10.1124/jpet.112.192138

45. Kobayashi S, Kajino S, Takahashi N, Kanazawa S, Imai K, Hibi Y, Ohara H, Itoh M, Okamoto T. 53BP2 induces apoptosis through the mitochondrial death pathway. Genes Cells. 2005 Mar;10(3):253-60. doi: 10.1111/j.1365-2443.2005.00835.x. PMID: 15743414.

46. , A. U. (2021, August 26). *ETAS®: Amino up functional ingredients*. ETAS® Standardized extract of asparagus stem. Retrieved May 23, 2022, from https://aminoup.info/en/etas

47. Hasegawa, T., Yoshida, S., Sugeno, N., Kobayashi, J., & Aoki, M. (1AD, January 1). *DNAJ/HSP40 family and parkinson's disease*. Frontiers. Retrieved May 25, 2022, from https://www.frontiersin.org/articles/10.3389/fnins.2017.00743/full

48. Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, Simonovic M, Doncheva NT, Morris JH, Bork P, Jensen LJ, Mering CV. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res. 2019 Jan 8;47(D1):D607-D613. doi: 10.1093/nar/gky1131. PMID: 30476243; PMCID: PMC6323986.

49. von Mering C, Huynen M, Jaeggi D, Schmidt S, Bork P, Snel B. STRING: a database of predicted functional associations between proteins. Nucleic Acids Res. 2003 Jan 1;31(1):258-61. doi: 10.1093/nar/gkg034. PMID: 12519996; PMCID: PMC165481.

50. Jensen LJ, Lagarde J, von Mering C, Bork P. ArrayProspector: a web resource of functional associations inferred from microarray expression data. Nucleic Acids Res. 2004 Jul 1;32(Web Server issue):W445-8. doi: 10.1093/nar/gkh407. PMID: 15215427; PMCID: PMC441545.

51. Zarouchlioti C, Parfitt DA, Li W, Gittings LM, Cheetham ME. DNAJ Proteins in neurodegeneration: essential and protective factors. Philos Trans R Soc Lond B Biol Sci. 2018 Jan 19;373(1738):20160534. doi: 10.1098/rstb.2016.0534. PMID: 29203718; PMCID: PMC5717533.

52. Mayer MP. Hsp70 chaperone dynamics and molecular mechanism. Trends Biochem Sci. 2013 Oct;38(10):507-14. doi: 10.1016/j.tibs.2013.08.001. Epub 2013 Sep 5. PMID: 24012426.

53. Wang H, Tan MS, Lu RC, Yu JT, Tan L. Heat shock proteins at the crossroads between cancer and Alzheimer's disease. Biomed Res Int. 2014;2014:239164. doi: 10.1155/2014/239164. Epub 2014 Jul 24. PMID: 25147790; PMCID: PMC4131517.

54. Campanella C, Pace A, Caruso Bavisotto C, Marzullo P, Marino Gammazza A, Buscemi S, Palumbo Piccionello A. Heat Shock Proteins in Alzheimer's Disease: Role and Targeting. Int J Mol Sci. 2018 Sep 1;19(9):2603. doi: 10.3390/ijms19092603. PMID: 30200516; PMCID: PMC6163571.

55. Repalli J, Meruelo D. Screening strategies to identify HSP70 modulators to treat Alzheimer's disease. Drug Des Devel Ther. 2015 Jan 7;9:321-31. doi: 10.2147/DDDT.S72165. PMID: 25609918; PMCID: PMC4294646.

56. Liu Q, Liang C, Zhou L. Structural and functional analysis of the Hsp70/Hsp40 chaperone system. Protein Sci. 2020 Feb;29(2):378-390. doi: 10.1002/pro.3725. Epub 2019 Oct 7. PMID: 31509306; PMCID: PMC6954727.