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Parkinsonism Quality Control in Mitochondria Determined by Real-Time Reverse Transcription Polymerase Chain Reaction

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Abstract

The primary causes of mitochondrial disease are nuclear DNA mutations, mitochondrial DNA mutations, combined nuclear and mitochondrial DNA defects, and random events. A prominent indicator of Parkinsonism is loss of black pigmentation. It falls to less than 80% due to the substantia nigra's loss of dopaminergic neurons. There was a correlation between the amount of black pigmentation loss and the degree of motor impairment.

Since reverse transcriptase was utilized to produce cDNA, reverse transcription is done after RNA extraction. A microliter of total RNA supplemented with nuclease-free, random hexamers, IORT Buffer, and Multi-Scribe Reverse Transcriptase was utilized to produce cDNA. In a thermal cycler, the reaction was run for the following durations: 10 minutes at 25°C, 120 minutes at 37°C, 5 seconds at 85°C, and finally 4°C.

The information was presented as a v-value, indicating the degree of enrichment of the target gene relative to the reference gene. Previous studies with mice injected with functional HtrA2/Omi protease activity showed comparable results, such as increased mtDNA deletions leading to early brain cell aging, reduced mitochondrial function, and induced up-regulation of CHOP.

These results show that the cell viability of the genotypes exhibits a dmg concentration-dependent effect, indicating that cellular content depletion may be caused by high content ratios of drug treatment with 6-OHDA. CHOP is up-regulated following treatment with 6-OHDA neurotoxin. Previous studies conducted in vitro have demonstrated that treatment with a model based on 6-OHDA neurotoxin activates pathways associated with mitochondrial stress, which further promotes neuronal death. In rat brain cells, 6-OHDA reversibly inhibited the activities of complexes I and IV, resulting in dopaminergic neurodegeneration—a characteristic that distinguishes PD pathogens. Consequently, the quantity of living cells will directly correspond with the concentration of dmg, thereby corroborating the findings of this investigation.

When compared to WT cells, real-time PCR can typically show that the deletion of HtrA 2 significantly upregulates the expression of Hsp60. This may be related to a lower threshold for mitochondrial stress in HtrA2 KO cells, which would account for the significant up-regulation of Hsp60 in HtrA2 KO cells after ADEP4 and ACP5 treatment, in comparison to WT cells.

Key words:

RT: real time, PCR: polymerase chain reaction, WT: wild type, dNTP: deoxynucleotide triphosphate

Introduction

Dopaminergic neurons heavily depend on proper mitochondrial homeostasis for their survival and function. It is commonly acknowledged that one of the main pathogenic pathways underlying the development of Parkinson's disease (PD) is mitochondrial dysfunction (1). In addition to helping with proper protein folding and preventing the accumulation of dangerous oxygen species, mitochondrial matrix also inhibits the aggregation of polypeptides. The mitochondrial unfolded protein response, or mtUPR, is a type of transcriptional response program that is represented by this molecular quality control mechanism (2). With the least amount of ROS released, fusion and fission dynamics maintain normal mitochondrial morphology and functions. Due to restrictions on mitochondrial motility, overexpression of the fusion and fission processes reduces energy production. This raises persistent oxidative stress, which encourages cell dysfunction and ultimately cell death. When mitochondrial quality control is compromised, damaged mitochondria accumulate, which raises the quantity of reactive oxygen species that contribute to dopaminergic neurodegeneration (3).

A highly conserved mitochondrial localized serine protease belonging to the high-temperature requirement factor A (HtrA) family is encoded by HtrA2, also referred to as Omi. HtrA2 proteases can activate or coordinate a variety of signaling pathways linked to PQC because of their structural conformation. When HtrA2 proteolytic activity is compromised, unfolded protein aggregation occurs within the mitochondria, mitochondrial respiration is hampered, and ROS product ions are increased, which causes the mitochondrial bioenergetics to become out of balance (4).

Dopaminergic Neuron Anatomy

The frontal cortex, limbic system, and hypothalamus—all of which are involved in movement coordination—have the highest concentration of dopamine in the corpus striatum. Cell bodies in Substantia Nigra that project to the striatum are known as the Nigrostriatal Pathway. Beginning in the ventral tegmental area, the mesolimbic pathway is a mesocortical pathway that connects the frontal cortex to the limbic system's amygdala and nucleus accumbens. Pituitary gland is controlled by the tuberohypophyseal pathway.

Postural tremors with amplitude between 8 and 10 Hz are less noticeable. It manifests during posture or action and continues to move **(figure 1).**

Figure 1. Resting tremors at 4-6 Hz usually start in the finger, usually the thumb

Materials and methods

(Forward primer: 5-AACTGAAAGAG AAGCAGAACCA-3; reverse primer: 5-TGTCTCAT AACCTTCTGAAAGTGC-3); mouse Hsp 60 (Forward primer: *5*-GCCTTAATGCTTCAAGGTGTAGA-3; reverse primer: *5*-CCCCATCTTTTGTTACTTTGGGA-3). The data was expressed as a v-value, which shows how much the target gene is enriched in comparison to the reference gene (5). Human HTRA2 mitochondrial isoform 1 preproprotein accession number [Homo sapiens]: NM_013247.5

The gene sequence is
CTACTGTCCGCCTGCTCGCGTCCTGGGTGCCGCCTCTGAGTAGGGCGGGC
GAGGAGGCAGCCAAGGCGGAGCTGATGGCTGCGCCGAGGGCGGGGCGGG
GTGCAGGCTGGAGCCTTCGGGCATGGCGGGCTTTGGGGGGCATTCGCTGG
GGGAGGAGACCCCGTTTGACCCCTGACCTCCGGGCCCTGCTGACGTCAGG
AACTTCTGACCCCCGGGCCCGAGTGACTTATGGGACCCCCAGTCTCTGGG
CCCGGTTGTCTGTTGGGGTCACTGAACCCCGAGCATGCCTGACGTCTGGG
ACCCCGGGTCCCCGGGCACAACTGACTGCGGTGACCCCAGATACCAGGAC
CCGGGAGGCCTCAGAGAACTCTGGAACCCGTTCGCGCGCGTGGCTGGCGG
TGGCGCTGGGCGCTGGGGGGGCAGTGCTGTTGTTGTTGTGGGGCGGGGGT
CGGGGTCCTCCGGCCGTCCTCGCCGCCGTCCCTAGCCCGCCGCCCGCTTCT
CCCCGGAGTCAGTACAACTTCATCGCAGATGTGGTGGAGAAGACAGCACC
TGCCGTGGTCTATATCGAGATCCTGGACCGGCACCCTTTCTTGGGCCGCGA
GGTCCCTATCTCGAACGGCTCAGGATTCGTGGTGGCTGCCGATGGGCTCA
TTGTCACCAACGCCCATGTGGTGGCTGATCGGCGCAGAGTCCGTGTGAGA
CTGCTAAGCGGCGACACGTATGAGGCCGTGGTCACAGCTGTGGATCCCGT
GGCAGACATCGCAACGCTGAGGATTCAGACTAAGGAGCCTCTCCCCACGC
TGCCTCTGGGACGCTCAGCTGATGTCCGGCAAGGGGAGTTTGTTGTTGCC
ATGGGAAGTCCCTTTGCACTGCAGAACACGATCACATCCGGCATTGTTAG
CTCTGCTCAGCGTCCAGCCAGAGACCTGGGACTCCCCCAAACCAATGTGG
AATACATTCAAACTGATGCAGCTATTGATTTTGGAAACTCTGGAGGTCCCC
TGGTTAACCTGGATGGGGAGGTGATTGGAGTGAACACCATGAAGGTCACA
GCTGGAATCTCCTTTGCCATCCCTTCTGATCGTCTTCGAGAGTTTCTGCATC
GTGGGGAAAAGAAGAATTCCTCCTCCGGAATCAGTGGGTCCCAGCGGCGC
TACATTGGGGTGATGATGCTGACCCTGAGTCCCAGCATCCTTGCTGAACTA
CAGCTTCGAGAACCAAGCTTTCCCGATGTTCAGCATGGTGTACTCATCCAT
AAAGTCATCCTGGGCTCCCCTGCACACCGGGCTGGTCTGCGGCCTGGTGA
TGTGATTTTGGCCATTGGGGAGCAGATGGTACAAAATGCTGAAGATGTTT
ATGAAGCTGTTCGAACCCAATCCCAGTTGGCAGTGCAGATCCGGCGGGGA
CGAGAAACACTGACCTTATATGTGACCCCTGAGGTCACAGAATGAATAGA
TCACCAAGAGTATGAGGCTCCTGCTCTGATTTCCTCCTTGCCTTTCTGGCT
GAGGTTCTGAGGGCACCGAGACAGAGGGTTAAATGAACCAGTGGGGGCA
GGTCCCTCCAACCACCAGCACTGACTCCTGGGCTCTGAAGAATCACAGAA
ACACTTTTTATATAAAATAAAATTATACCTAGCAACATATTATAGTAAAAA
ATGAGGTGGGAGGGCTGGATCTTTTCCCCCACCAAAAGGCTAGAGGTAAA

GCTGTATCCCCCTAAACTTAGGGGAGATACTGGAGCTGACCATCCTGACC
TCCTATTAAAGAAAATGAGCTGCTGCCA

DNA damage-inducible transcript 3 protein [Mus musculus]
NCBI Reference Sequence: NM_001290183.1

GGTCAGTTATCTTGAGCCTAACACGTCGATTATATCATGTTGAAGATGAGC
GGGTGGCAGCGACAGAGCCAGAATAACAGCCGGAACCTGAGGAGAGAGA
ACCTGGTCCACGTGCAGTCATGGCAGCTGAGTCCCTGCCTTTCACCTTGGA
GACGGTGTCCAGCTGGGAGCTGGAAGCCTGGTATGAGGATCTGCAGGAG
GTCCTGTCCTCAGATGAAATTGGGGGCACCTATATCTCATCCCCAGGAAA
CGAAGAGGAAGAATCAAAAACCTTCACTACTCTTGACCCTGCGTCCCTAG
CTTGGCTGACAGAGGAGCCAGGGCCAACAGAGGTCACACGCACATCCCA
AAGCCCTCGCTCTCCAGATTCCAGTCAGAGTTCTATGGCCCAGGAGGAAG
AGGAGGAAGAGCAAGGAAGAACTAGGAAACGGAAACAGAGTGGTCAGT
GCCCAGCCCGGCCTGGGAAGCAACGCATGAAGGAGAAGGAGCAGGAGAA
CGAGCGGAAAGTGGCACAGCTAGCTGAAGAGAACGAGCGGCTCAAGCAG
GAAATCGAGCGCCTGACCAGGGAGGTGGAGACCACACGGCGGGCTCTGA
TCGACCGCATGGTCAGCCTGCACCAAGCATGAACAGTGGGCATCACCTCC
TGTCTGTCTCTCCGGAAGTGTACCCAGCACCATCGCGCCAGCGCCAAGCA
TGTGACCCTGCACTGCACTGCACATGCTGAGGAGGGGACTGAGGGTAGAC
CAGGAGAGGGCTCGGCTTGCACATAGACGGTACATTGTTTATTACTGTCC
ATGTCCAGTAAAGTGACTTTGTGTCAACAAAAAAAAAAAAAAAAA

Using the Thermo Fisher Scientific Vybran MTT Cell Proliferation Assay Kit, 30 µL of MTT solution was added and incubated for two hours following drug treatment. To make the 12 mM MTT reagent, dissolve 15 mg of MTT in 30 ml of media and add 3 mL of PBS. Following the removal of the medium, the cells were again suspended in 100 µL of DMSO and incubated for ten minutes at 37° C. At 570 nm wavelength, the absorbance was measured with a SpeclraMax (MS) spectrophotometer.

Results

Real-Time RT-PCR

RT-PCR analysis demonstrated that, in comparison to WT cells, depletion of HtrA 2 causes a significant up-regulation of Hsp60 expression. This may be associated with a decrease in the threshold for mitochondrial stress in HtrA2 KO cells, which would result in an up-regulation of Hsp60 that is significantly lower in HtrA2 KO cells than in WT cells after ADEP4 and ACP5 treatment.

Discussion

The cell viability of the genotypes exhibits a dmg concentration-dependent effect in these results, suggesting that high content ratios of drug treatment with 6-OHDA may be the cause of cellular content depletion. After 6-OHDA neurotoxin treatment, CHOP is up-regulated (6). Prior in vitro research has shown that treatment with a model based on the 6-OHDA neurotoxin causes the activation of mitochondrial stress pathways, which in turn promotes the death of neurons. 6-OHDA reversibly inhibited complexes I and IV activities in rat brain cells, causing dopaminergic neurodegeneration, a characteristic of PD pathogens (7). As a result, the number of living cells will directly correlate with the dmg concentration, supporting the results of the current study.

Mitochondrial disease has the following primary causes:

• Mutations in mitochondrial DNA (inherited or acquired)

• Mutations in nuclear DNA (inherited or acquired)

• Defects in both mitochondrial and nuclear DNA combined

• Incidents at random

Mutations occur in the mtDNA

Heteroplasmy is the term for a mixed population of wild-type and mutant mtDNA within a single cell. A skewed population of wild-type or mutant mtDNA is produced when heteroplasmic cells divide and the mtDNA is randomly distributed to the daughter cells. Different amounts of mutant mtDNA are present in daughter cells as a result of random mtDNA segregation during mitosis. The clinical phenotype is determined by the degree of heteroplasmy.

Conclusions

Real-time PCR can generally demonstrate that the absence of HtrA2 results in a significant up-regulation of Hsp60 expression relative to WT cells. This could be linked to a decreased threshold for mitochondrial stress in HtrA2 KO cells, which would explain why, following ADEP4 and ACP5 treatment, Hsp60 is significantly up-regulated in HtrA2 KO cells compared to WT cells.

The transcription factor CHOP controls the signaling associated with mitochondrial stress in cells, and in these cells, treatment with ADEP4 and ACP5 completely removes the up-regulation of Hsp60. The levels of Atg5 suggest that both the loss of HtraA2 and the loss of CHOP appear to have a substantial effect on the transcriptional activation of mitophagy.

References

1- **Luo, Y. et al.** (2015). Mitochondria: A therapeutic target for Parkinson's disease? ', *International Journal of Molecular Sciences*, 16(9), pp. 20704-20730. doi:10.3390/ijms 16 0920704.
2- **Held, N. M. and Houtk Ooper.** (2015). ' Mitochondrial quality control pathways as determinants of metabolic health, *Bioassays*, 37(8), pp. 867-876. doi: 10.1002 /bies
3- **Kotiadis, V. N., Duchen, M. R. and Osellame, L. D.** (2014). Mitochondrial quality control and communications with the nucleus are important in maintaining mitochondrial function and cell health', *Biochemical Biophysicsa Acta - General Subjects*. The Authors, 1840(4), pp. 125-4- 1265. doi: 10. 1016 /j.bbagen .2013 .10.041.
4- **Quiros, P. M., Langer, T. and Lopez-Olin, C.** (20l5). ' New roles for mitochondrial proteases in health, ageing and disease, *Nature Reviews 1\40 /molecular Cell Biology*. Nature Publishing Group, 16(6), pp. 345- 359. doi: 10.1038/nnn3984.
5- **Zucker, B. et al.**(2005). ' Transcription al dysregulation in striatal projection- and inteneurons in a mouse model of Huntington's disease: Neuronal selectivity and potential Neuro-protective role of HAP1 ', Human A10 /cellular Genetics, 14 (2), pp. 179- 189. doi:10.1093/hm g/dd 2014.
6- **Coppola-Segovia, V. *et al.*** (2017). ' ER Stress Induced by Tunicamycin T riggers u-Synucle in Oligomerization, Dopaminergic Neurons Death and Locomotor Impairment: a New Model of Parkinson's Disease.*Neurobiology*. Molecular Neurobiology, 54(8), pp. 5798- 5806. doi: 10. 1007 / s l2035- 016-0114-x.
7- **Requejo-Aguilar, R. and Bolanos, J. P.** (2016). ' Mitochondrial control of cell bioenergetics in Parkinson's disease.' *Free radical biology & medicine*, JOO, pp. 1- 15. doi:10.1016/j.fre.eradbiome d.2016.04.012.