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Proteomic Insights into the Protective Mechanisms of an *In Vitro* Oxidative Stress Model of Early Stage Parkinson's Disease

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Abstract

Previous studies in Parkinson's disease (PD) models suggest that early events along the path to neurodegeneration involve activation of the ubiquitin-proteasome system (UPS), endoplasmic reticulum-associated degradation (ERAD), and the unfolded protein response (UPR) pathways, in both the sporadic and familial forms of the disease, and thus ER stress may be a common feature. Furthermore, impairments in protein degradation have been linked to oxidative stress as well as pathways associated with ER stress. We hypothesize that oxidative stress is a primary initiator in a multi-factorial cascade driving dopaminergic (DA) neurons towards death in the early stages of the disease. We now report results from proteomic analysis of a rotenone-induced oxidative stress model of PD in the human neuroblastoma cell line, SH-SY5Y. Cells were exposed to sub-micromolar concentrations of rotenone for 48 hours prior to whole cell protein extraction and shotgun proteomic analysis. Evidence for activation of the UPR comes from our observation of up-regulated Binding immunoglobulin Protein (BiP), heat shock proteins, and foldases. We also observed up-regulation of proteins that contribute to the degradation of misfolded or unfolded proteins controlled by the UPS and ERAD pathways. Activation of the UPR may allow neurons to maintain protein homeostasis in the cytosol and ER despite an increase in reactive oxygen species due to oxidative stress, and activation of the UPS and ERAD may further augment clean-up and quality control in the cell.

Keywords

Oxidative stress; Parkinson's disease; Rotenone; Proteomics; Unfolded protein response

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Introduction

Parkinson's disease (PD) is a complex, progressive neurodegenerative movement disease that results primarily from the death of dopaminergic (DA) neurons in the *substantia nigra pars compacta*. Although the cause of sporadic PD is unknown, epidemiological studies suggest cooperation with environmental toxins, notably mitochondrial complex I inhibitors and gene mutations [18]. Oxidative stress is a leading theory of the pathogenesis of PD, supported by analysis of postmortem PD brains [33], and by recapitulation of the oxidative damage seen in PD by low-grade, chronic inhibition of complex 1 with rotenone, both *in vivo* and *in vitro* [2,10]. The common feature associated with oxidative stress and subsequent neurodegeneration is endoplasmic reticulum (ER) stress: a disturbance in the ability of the ER to process and/or fold proteins. The resulting accumulation of misfolded proteins will elicit the Unfolded Protein Response (UPR), leading to transcription of chaperones, foldases, ER-associated degradation (ERAD) machinery and antioxidants. Acute activation of the UPR is beneficial in responding to transient stress. However, if ER stress is chronic, or if protective measures are incapable of maintaining ER homeostasis, the UPR activates apoptotic pathways to avoid damage to neighboring cells [32]. Our goal in this study was to produce an early-stage model of PD by using very small doses of the oxidizing toxin rotenone and report on the changes in the whole cell proteome.

Materials & Methods

Cell Culture

Human neuroblastoma cells (SH-SY5Y) were obtained from the American Type Culture Collection (Rockville, MD).

Cell viability

Cell viability was determined by a MTT (3-(4, 5-dimethylthiazol)-2,5diphenyltetrazolium bromide) assay following the manufacture's guidelines (ATCC catalog # 30-1010K).

Proteasome Activity Assay - Chymotrypsin-like activity

Control and rotenone treated cells were incubated with Succinyl-Leu-Leu-Val-Tyr-7-amino-4-methylcoumarin (LLVY-MCA) (obtained from Dr. M. Gaczynska, Univ. of Texas Health Science Center, San Antonio) at 37°C for 1 hour. Fluorescence of released MCA was measured at 460 nm (with 380 nm excitation).

ssDNA Apoptosis

Control and rotenone-treated cells were analyzed for apoptosis using a Millipore ssDNA Apoptosis ELISA kit (APT225) in accordance with the manufacture's guidelines.

Caspase-3 Activity Assay

Caspase-3 activity was quantified using Ac-DEVD-AMC Caspase -3 fluorogenic substrate (BD Pharmingen) in accordance with the manufacture's guidelines.

Sample Preparation

Cells were prepared for protein identification by incubation for 48 hours at 37°C with 5nM, 10nM, 20nM, 50nM, and 100nM rotenone, or as a control, vehicle only. Proteins from cell culture monolayers were extracted by adding 500μL buffer (25mM Tris-HCl pH 7.6, 150mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS) and 10μL Protease Inhibitor Cocktail (Sigma P8340) to 10^6 cells. After 30 min lysis at 4°C, cells were centrifuged and the supernatant was stored at −20°C. Total protein was quantified using the

Micro BCA kit (Pierce) following manufacture's protocol. Proteins were carbamidomethylated and precipitated with acetone [37] prior to trypsin digestion (Promega Gold, mass spectrometry grade) (1:20 protein:enzyme). Capillary liquid chromatography-tandem mass spectrometry (LC/MS/MS) was performed with a splitless nanoLC-2D pump (Eksigent), a 50 μm-i.d. column packed with 10 cm of 5 μm-i.d. C18 particles, and a linear ion trap tandem mass spectrometer (LTQ-XLS; ThermoFisher, San Jose, CA), where the top 7 eluting ions were fragmented by collision-induced dissociation (CID). The capillary LC gradient was 2 to 98% 0.1% formic acid/acetonitrile over 60 min at a flow rate of 300 nL/min. Probability-based and error-tolerant protein database searching of MS/MS spectra against the NCBI non-redundant human protein database was performed with a 10-node MASCOT cluster (ver. 2.1). Search criteria included: peak picking with Xcalibur (ver. 2.0.6; ThermoFisher); 1000 ppm precursor ion mass tolerance, 0.8 Da product ion mass tolerance, 3 missed cleavages, trypsin, carbamidomethyl cysteines and oxidized methionines as variable modifications, and an ion score threshold of 20.

Criteria for Protein Identification

Scaffold (version scaffold_2_06_00, Proteome Software Inc., Portland, OR) was used to validate MS/MS based peptide and protein identifications. Peptide identifications were accepted if they could be established at greater than 90.0% probability (Peptide Prophet algorithm [15]). Protein identifications were accepted if they could be established at a greater than 95.0% probability (Protein Prophet algorithm [26]) and contained at least 1 identified peptide.

Results & Discussion

In order to analyze the proteome of early-stage PD, we sought to identify a concentration range of rotenone in which cultured neuroblastoma cells are viable and are not apoptotic after 48 hours. We evaluated the results of three different measurements: cellular viability/mitochondrial activity, apoptosis activity assessed by single-stranded DNA (ssDNA), and apoptosis activity assessed by caspase-3 activity. SH-SY5Y cells were incubated with submicromolar concentrations of rotenone for 48 hours. Mitochondrial activity (MTT assay, Fig.1) was unaffected at a rotenone concentration ≤ 50 nM ($p \leq 0.05$). However, when the concentration of rotenone reached ≥100 nM, a significant decrease in cell viability was seen (100nM: p=.0001; 100nM-1μM: p=.0006). The ssDNA apoptosis assay showed no significant increase in apoptosis (Fig.2). Furthermore, the caspase-3 activity showed a significant increase at 50 nM and 100 nM (p= .043 and p= .014, respectively) (Fig. 3). Approximately a 2.5 fold increase in activity was seen, which we estimate may only represent 25% of the maximal caspase-3 activation [21]. The increase in caspase-3 activity at 50 nM and 100 nM rotenone does not correlate with an increase in ssDNA apoptosis at the same concentrations, suggesting that compensatory factors were released by the cell to counteract the apoptotic cascade, potentially through UPR activation and subsequent production of anti-apoptotic proteins. Therefore, we chose the unaffected range (5nM, 10nM, 20nM, and 50nM) of rotenone concentrations to look for the initial response of the cells to oxidative stress. This time point and concentration range may represent the earliest phase in the progressive development of Parkinson's-like neurodegeneration. We also used exposure to 100nM rotenone to examine the proteome in cells displaying decreased viability.

In control SH-SY5Y cells, we identified tryptic peptides from 242 proteins with high probability, including proteins that are related to the UPR and ER stress pathways that are listed in Table 1. Three heat-shock proteins were identified: HSP90 (both the cytosolic HSP90α and ER HSP90β forms), GRP75, and HSP60. GRP75, in concert with HSP60, is thought to participate in the refolding of proteins translocated into the mitochondria [17,20].

Additionally, some components involved in the UPS system were identified: the ubiquitin carboxyl-terminal hydrolase enzyme, and a ubiquitin E1 activating enzyme. Both proteins are vital to the function of the UPS and both have been found to be dysfunctional in moderate to late stages of PD [23]. Finally, glutathione S-transferase (GST), a major player in the detoxification and protection against oxidizing toxins, was identified in the control group [6]. This constitutive expression of stress-related proteins in dopaminergic neurons suggests that these neurons have adapted to the endogenous stress associated with dopamine metabolism and subsequent production of reactive oxygen species (ROS) [1]. In other words, neurons may rely on their constitutive levels of HSPs as a preventative mechanism of defense against protein misfolding induced by stressful factors or those that are associated with neurodegenerative diseases [4].

At 5nM, 10nM, 20nM, and 50nM concentrations of rotenone we identified tryptic peptides from 545 proteins with high probability, including proteins that are related to survival, growth, and protection (Table 1). Of great interest, at 5nM and 10nM, DJ-1 was identified. DJ-1 is classically associated with PD by deletion and point mutations shown to be responsible for the onset of familial PD [34]. We also detected BiP, starting at 10nM and continuing through 100nM. The expression of BiP indicates the activation of the UPR [32]. BiP can act to protect cells from oxidative stress. This stress can cause partial unfolding or aggregation of proteins. However, BiP can temporarily bind to hydrophobic residues exposed by ROS, thereby allowing the protein to potentially refold and/or preventing protein aggregation [25].

UPR activation has been shown to increase the expression of multiple chaperones, foldases, and components of the UPS and ERAD system [32]. This increase is clearly evident in Table 1. A recent immunohistochemistry study showed an increase in ER-resident chaperones in PD brain [35], suggesting that our results are relevant to human pathology. To validate an increase in UPS and ERAD components, we assayed the chymotrypsin-like activity of the 20S proteasome (Figure 4). From 20nM to 100nM rotenone, a significant increase in the activity of the chymotrypsin-like activity is seen ($p = .000012$) compared to the lower concentrations of rotenone and control. This suggests that the UPR has increased the expression of proteasome components necessary to handle the increased burden of misfolded/unfolded proteins due to the increase in oxidative stress. Also, of interest is the identification of the transitional ER ATPase at the 20nM and 100nM group. This protein regulates E3 ligase activity and may be required for export of misfolded proteins from the ER to be degraded by the proteasome. Of further importance, we identified two, components of the mitochondrial permeability transition pore (mPTP): Voltage-Dependant Anion Channel (VADC) 1 and 2. It has been shown recently that VDAC1 acts as a mitochondrial target of Parkin-mediated poly-ubiquitin chains and is therefore necessary for PINK1/Parkin-directed autophagy of damaged mitochondria [8]. Specifically it has been shown that with a decrease in mitochondrial membrane potential, Parkin translocates to the mitochondria in response to ROS. So with VDAC1 acting as a mitochondrial target of Parkin-mediated poly-ubiquitin chains, a Parkin-dependent mitophagic clearance could arrest the release of pro-apoptotic factors from damaged mitochondria [8]. So an increase in VDAC1 can aid in the removal of damaged mitochondria by assisting damaged mitochondria into forming autophagosomes.

At higher rotenone concentrations, we found several proteins that are involved in cytoskeletal remodeling and development of neurites (Stathmin1, Stathmin2, Neuron Navigator 2), as well as maintaining mitochondrial homeostasis (Prohibitin1, Prohibitin2) [27,19,30].

The UPR activation and its related products are able to keep the neurons healthy enough to sustain viability and overcome the induced stress up to a point. It has been clearly shown in past studies that with sustained UPR activation, apoptosis occurs [32,13,18]. Previous studies showed activation of the UPR after oxidative stress was induced in neuronal cells [12,31] using high toxin concentrations that resulted in 50% cell death within 24-hours. We observed similar effects with 100nM rotenone, which significantly decreased cell viability (Fig. 1), increased caspase-3 activity (Fig. 3), and increased cytochrome c (Table 1). We believe this marks a threshold of defense that the neurons are capable of producing.

Our results indicate that SH-SY5Y cells activate the UPR acutely at rotenone doses that the cells can survive. As the concentration of rotenone increased so did the expression of proteins necessary to handle the accumulation of misfolded or unfolded proteins (Table 1). Much information exists about neurodegenerative disorders in the moderate to late stages of the diseases, where most of the outward symptoms manifest themselves. However, an understanding of the early stages of the progression towards apoptosis in PD can greatly assist research toward preventative therapies.

In conclusion, our whole cell proteomic analysis identified proteins that are involved in the protective UPR and ER stress pathways in dopaminergic neurons subjected to oxidative stress. Our results clearly show that these cells have the ability to overcome low-level oxidative damage, which might resemble the initial biochemical events of PD. However, with higher levels of stress, or prolonged stress, the protective pathways are insufficient to prevent the activation of apoptosis. The question remains whether the UPR and ER pathways can be harnessed and manipulated to provide a new early intervention strategy for treatment of PD and other neurodegenerative diseases.

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References

1. Berg, D. Redox imbalance. In: Qureshi, G.; Parvez, S., editors. Oxidative stress and neurodegenerative diseases. Elsevier; 2005. p. 183-191.
2. Cannon J, Tapias V, Na H, Honick A, Drolet R, Greenamyre JT. A highly reproducible rotenone model of Parkinson's disease. Neurobiology of Disease. 2009; 34:279–290. [PubMed: 19385059]
3. Chen S, Smith D. Hop as an adaptor in the heat shock protein 70 (HSP70) and HSP90 chaperone machinery. J. Biol. Chem. 1998; 273:35194–35200. [PubMed: 9857057]
4. Cheng S, Brown I. Neuronal expression of constitutive heat shock proteins: implications for neurodegenerative disorders. Cell Stress & Chaperones. 2007; 12:51–58. [PubMed: 17441507]
5. Csermely P, Schainder T, Sȍti C, Prohászka Z, Nardai G. The 90-kDa molecula chaperone family: structure, function, and clinical applications. A comprehensive review, Pharmacol. Ther. 1998; 79:129–168.
6. Douglas KT. Mechanisms of action of glutathione-dependent enzymes. Adv. Enzymol. 1987; 59:103–167. [PubMed: 2880477]
7. Frickel EM, Frei P, Bouvier M, Stafford W, Helenius A, Clockshuber R, Ellgaard L. ERp57 is a multifunctional thiol-disulfide oxioreductase. J. Biol. Chem. 2004; 279:18277–18287. [PubMed: 14871896]
8. Geisler S, Holmstrom KM, Skujat D, Fiesel FC, Rothfuss OC, Kahle PJ, Springer W. PINK1/PARKIN-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. Nature Cell Biology. 2009; 12:119–131.

9. Graves J, Metukuri M, Scott D, Rothermund K, Prochownik E. Regulation of reactive oxygen species homeostasis by peroxiredoxin and c-Myc. J. Biol. Chem. 2009; 284:6520–6529. [PubMed: 19098005]
10. Greenamyer JT, Betarbet R, Sherer TB. The rotenone model of Parkinson's disease: genes, environment and mitochondria. Parkinsonism and Related Disorders. 2003; 9:59–64.
11. Hohfeld J, Hartl FU. Role of the chaperonin cofactor HSP10 in protein folding and sorting in yeast mitochondria. J. Cell. Biol. 1994; 126:305–315. [PubMed: 7913473]
12. Holtz WA, Turetzky JM, Jong YI, O'Malley KL. Oxidative stress-triggered unfolded protein response is upstream of intrinsic cell death evoked by parkinsonian mimetics. J. Neurochem. 2006; 99:54–69. [PubMed: 16987235]
13. Hoozemans JJM, van Haastert ES, Eikelenboom P, de Vas RAI, Rozemuller JM, Sheper W. Activation of the unfolded protein response in Parkinson's disease. B.B.R.C. 2007; 354:707–711.
14. Imai J, Maruya M, Yashiroda H, Yahara I, Tanaka K. The molecular chaperone HSP90 plays a role in the assembly and maintenance of the 26S proteasome. EMBO J. 2003; 22:3557–3567. [PubMed: 12853471]
15. Keller A, Nesvizhskii AI, Kolker E, Aebersold R. Emperical statistical model to estimate the accuracy of peptide identification made by MS/MS and database search. Anal. Chem. 2002; 74:5383–5392. [PubMed: 12403597]
16. Kersey PJ, Duarte J, Williams A, Karavidopoulou Y, Birney E, Apweiler R. The International Protein Index: An integrated database for proteomics experiments. Proteomics. 2004; 4:1985–1988. [PubMed: 15221759]
17. Koll H, Guiard B, Rassow J, Ostermann J, Horwich A, Neupert W, Hartl FU. Antifolding activity of HSP60 couples protein import into the mitochondrial matrix with export to the intermembrane space. Cell. 1992; 68:1163–1175. [PubMed: 1347713]
18. Lin CY, Kaufman RJ. The unfolded protein response. Journal of Cell Science. 2003; 116:1861–1862. [PubMed: 12692187]
19. Maes T, Barcelo A, Buesa C. Neuron navigator: a human gene family with homology to unc-53, a cell guidance gene from Caenorhabditis elegans. Genomics. 2002; 80:21–30. [PubMed: 12079279]
20. Manning-Krieg UC, Scherer PE, Schatz G. Sequential action of mitochondrial chaperones in protein import into the matrix. The EMBO Journal. 1991; 10:3273–3280. [PubMed: 1915294]
21. Mashima T, Naito M, Kataoka S, Kawai H, Tsuruo T. Aspartate-based inhibitor of interleukin-1 beta-converting enzyme prevents antitumor agent-induced apoptosis in human myeloid leukemia U937 cell. B.B.R.C. 1995; 209:907–915.
22. McClung JK, Jupe ER, Liu XT, Dell'Orco RT. Prohibitin: potential role in senescence, development, and tumor suppression. Exp.Gerontol. 1995; 30:99–124. [PubMed: 8591812]
23. McNaught KSP, Olanow CW. Proteolytic stress: a unifying concept for the etiopathogenesis of parkinson's disease. Ann Neuro. 2003; 53:73–86.
24. Mehlen P, Hickey E, Weber LA, Arrigo AP. Large unphosphorylated aggregates as the active form of HSP27 which controls intracellular reactive oxygen species and glutathione levels and generates a protection against TNFalpha in NIH-3T3-ras cells. Biochem. Biophys. Res. Commun. 1997; 241:187–192. [PubMed: 9405255]
25. Morano K. New tricks for an old dog. The evolving world of HSP70. Ann. N.Y. Acad. Sci. 2007; 1113:1–14. [PubMed: 17513460]
26. Nesvizhskii AI, Keller A, Kolker E, Aebersold R. A statistical model for identifying proteins by tandem mass spectrometry. Anal. Chem. 2003; 75:4646–4685. [PubMed: 14632076]
27. Ozon S, El Mestikawy S, Sobel A. Differential, regional, and cellular expression of the Stathmin family transcripts in the adult rat brain. J. Neurosci. Res. 1999; 56:553–564. [PubMed: 10369222]
28. Parcellier A, Schmitt E, Gurbuxani S, Seigneurin-Berny D, Pance A, Chantome A, Plenchette S, Khochbin S, Solary E, Garrido C. HSP27 is a ubiquitin-binding protein involved in I-κBα proteasomal degradation. Mol. Cell. Biol. 2003; 23:5790–5802. [PubMed: 12897149]
29. Picklo M, Amarnath V, McIntyre J, Graham D, Montine T. 4-Hydroxy-2(*E*)-Nonenal inhibits CNS mitochondrial respiration at multiple sites. J. Neurochem. 1999; 72:1617–1624. [PubMed: 10098869]

30. Ross J, Nagy Z, Kirken R. The PHB1/2 phosphocomplex is required for mitochondrial homeostasis and survival of human T cells. J. Biol. Chem. 2008; 283:4699–4713. [PubMed: 18086671]

31. Ryu EJ, Harding HP, Angelastro JM, Vitolo OV, Ron D, Greene LA. Endoplasmic reticulum stress and the unfolded protein response in cellular models of Parkinson's disease. Journal of Neurosci. 2002; 22:10690–10698.

32. Schröder M, Kaufman R. The mammalian unfolded protein response. Annu. Rev. Biochem. 2005; 74:739–789. [PubMed: 15952902]

33. Sherer T, Betarbet R, Stout A, Lund S, Baptista M, Panov A, Cookson M, Greenmyre T. An *in vitro* model of Parkinson's Disease: linking mitochondrial impairment to altered α-synuclein metabolism and oxidative damage. Journal of Neurosci. 2002; 22:7006–7015.

34. Taira T, Saito Y, Niki T, Iguchi-Ariga SMM, Takahashi K, Ariga H. DJ-1 has a role in antioxidative stress to prevent cell death. EMBO reports. 2004; 5:213–218. [PubMed: 14749723]

35. Wilhelmus MMM, Verhaar R, Andringa G, Bol JGJM, Cras P, Shan L, Hoozemans JJM, Drukarch B. Presence of tissue transglutaminase in granular endoplasmic reticulum is characteristic of melanized neurons in Parkinson's disease brain. Brain Path. 2010 in press (doi:10.1111/j.1750-3639.2010.00429.x).

36. Wilkinson B, Gilbert H. Protein disulfide isomerase. Biochimica et biophysica acta. 2004; 1699:35–44. [PubMed: 15158710]

37. Wang N, Xu M, Wang P, Li L. Development of mass spectrometry-based shotgun method for proteome analysis of 500 to 5000 cancer cells. Anal. Chem. 2010; 82:2262–2271. [PubMed: 20092350]

38. Yaffe M, Farr GW, Miklos D, Horwich AL, Sternlicht ML, Sternlicht H. TCP1 complex is a molecular chaperone in tubulin biogenesis. Nature. 1992; 358:245–248. [PubMed: 1630491]

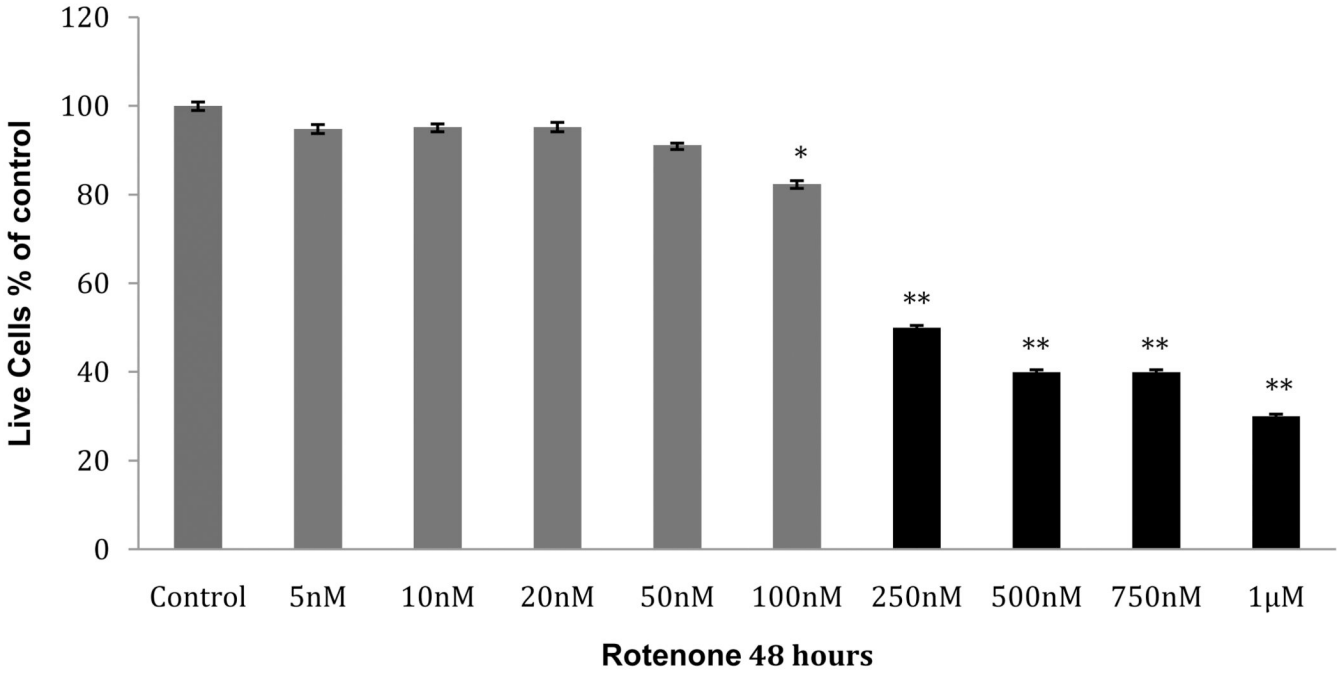

Fig. 1. MTT viability assay

MTT cell viability assay performed on SH-SY5Y cultures pre-incubated with various concentrations of rotenone for 48 hours. Gray bars indicate cells extracted for proteomic analysis (Table 1). Data (mean ± SEM) are expressed as % of control (no rotenone). Asterisk (*) indicates $p<0.0001$ by ANOVA: 100nM rotenone-treated compared to 50nM; double asterisk (**) indicates $p<0.0006$ by ANOVA: 250nM-1μM rotenone-treated compared to 5nM–50nM group.

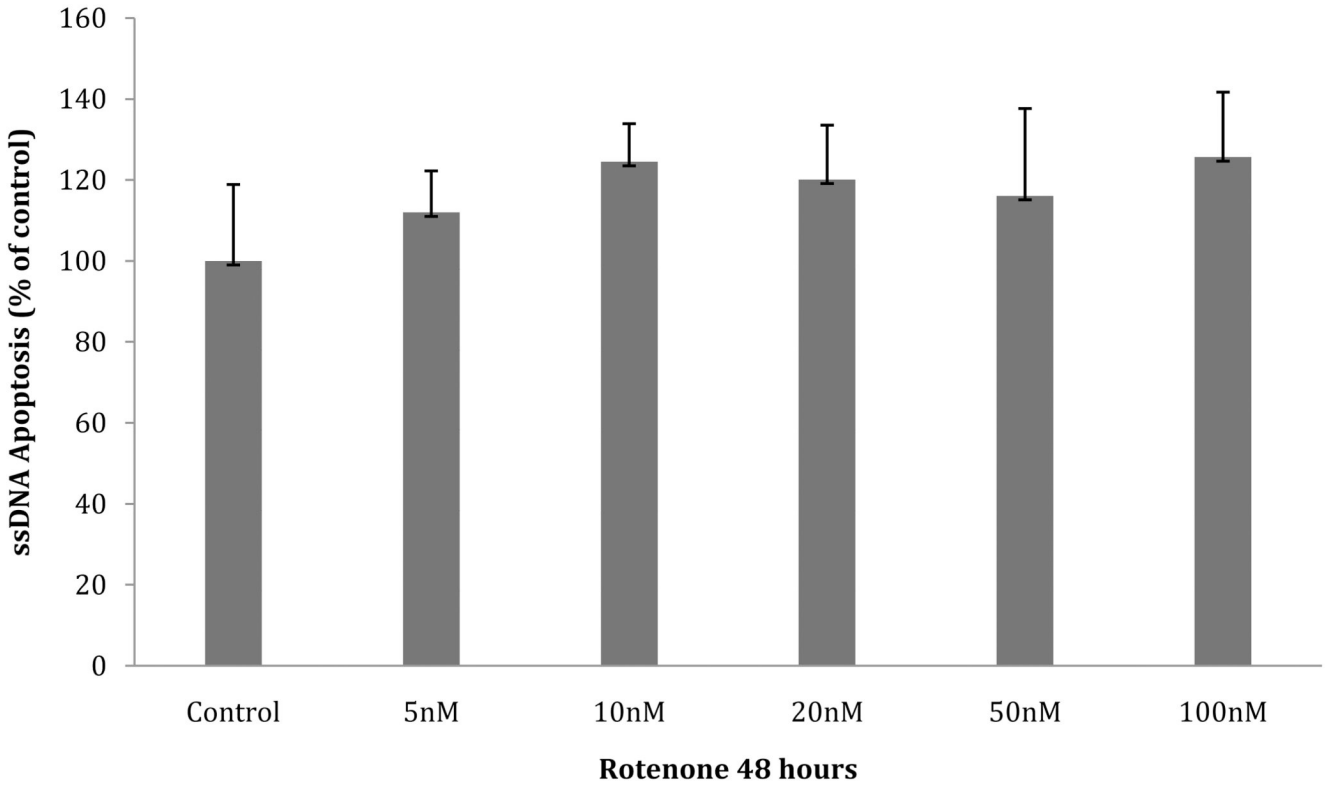

Fig. 2. ssDNA apoptotic assay (ELISA)

SH-SY5Y cultures pre-incubated with various concentrations of rotenone for 48 hours. Data (mean ± SEM) expressed as % of control (no rotenone).

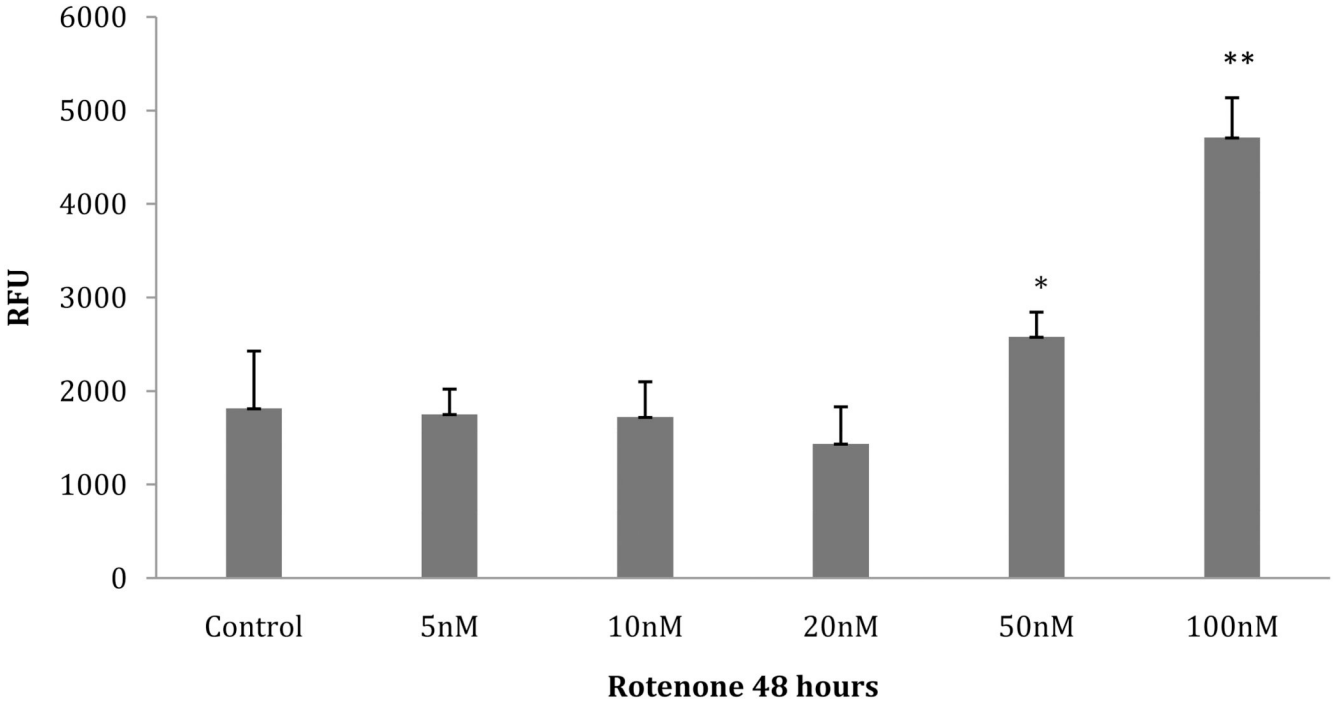

Fig. 3. Caspase-3 activity assay

Caspase-3 activity measured on SH-SY5Y cells pre-incubated with various concentrations of rotenone for 48 hours. Data expressed as relative fluorescence units (RFU). Asterisk (*) indicates p=0.043 by ANOVA: 50nM rotenone-treated compared to 5nM–20nM; double asterisk (**) indicates p=0.014 by ANOVA: 100nM rotenone-treated compared to 5nM–50nM rotenone group.

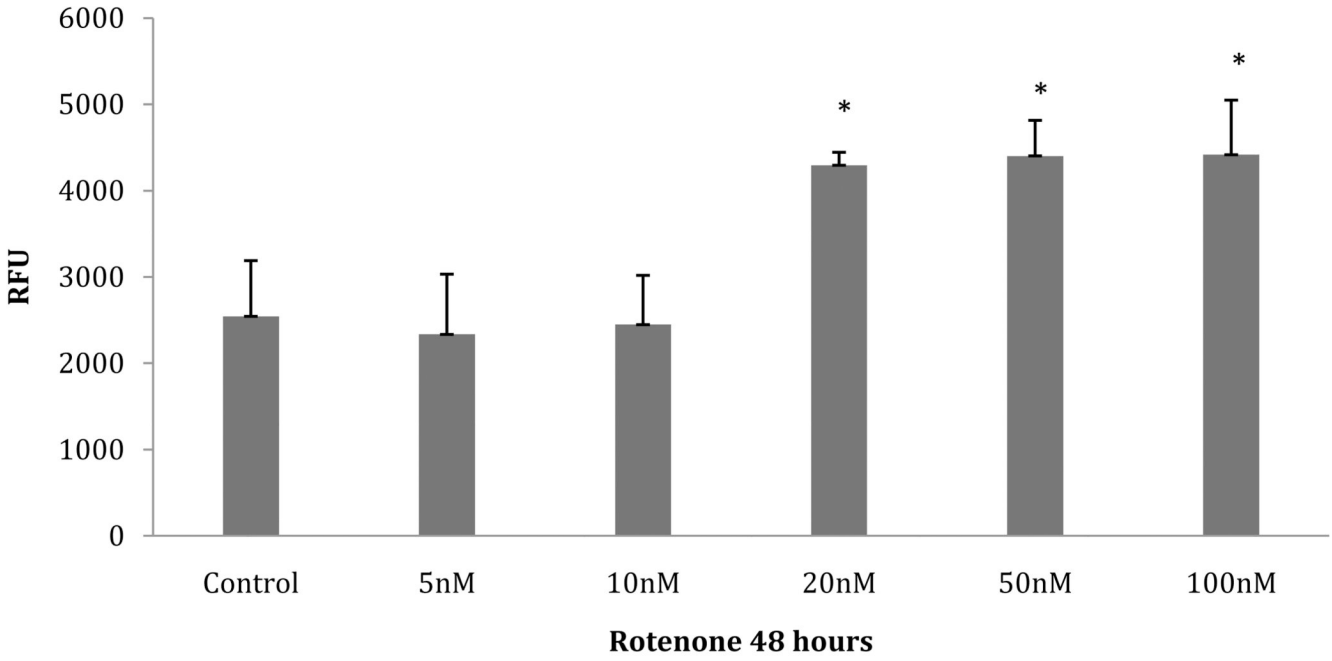

Fig. 4. Proteasome activity Assay

Fluorogenic substrate measured chymotrypsin-like activity in 20S proteasomes from SH-SY5Y cells exposed to various concentrations of rotenone for 48 hours. Data expressed as relative fluorescence units (RFU). Asterisk (*) indicates p=0.000012 by ANOVA: 20nM–100nM rotenone-treated group compared to control-10nM group.

Table 1
Identified proteins related to UPR/ER stress pathways following treatment with rotenone

Proteins listed with International Protein Index (IPI) [16], MASCOT score, and function. Protein not described in text listed with references to PD and neurodegeneration.

Rotenone 48 hrs	Function	Protein	Accession Numbers	Mascot Score
Control	chaperone/foldase	HSP90-beta [5,14]	IPI00414676	422
	chaperone/foldase	HSP90-alpha isoform 2 [5,14]	IPI00382470	288
	chaperone/foldase	GRP75	IPI00007765	90
	chaperone/foldase	HSP60, mitochondrial	IPI00784154	151
	chaperone/foldase	TCP1 T-complex subunit 3 isoform b [38]	IPI00290770	67
	chaperone/foldase	TCP1 T-complex protein 1 subunit alpha [38]	IPI00290566	75
	chaperone/foldase	TCP1 T-complex protein 1 subunit beta [38]	IPI00297779	202
	chaperone/foldase	Peptidyl-prolyl cis-trans isomerase A	IPI00419585	40
	UPS system	Ubiquitin carboxyl-terminal hydrolase isozyme	IPI00018352	192
	UPS system	Ubiquitin-like modifier-activating enzyme 1	IPI00645078	127
	antioxidant	Glutathione S-transferase	IPI00219757	132
5nM	chaperone/foldase	HSP90-beta [6,15]	IP00I414676	155
	chaperone/foldase	HSP60, mitochondria	IPI00784154	101
	chaperone/foldase	HSP75, mitochondria	IPI0030275	65
	chaperone/foldase	TCP1 T-complex protein 1 subunit zeta [38]	IPI00027626	53
	chaperone/foldase	Peptidyl-prolyl cis-trans isomerase A	IPI00419585	63
	chaperone/foldase	Prefoldin subunit 5	IPI00015361	93
	UPS system	Ubiquitin-like modifier activating enzyme 1	IPI00552452	33
	UPS system	Proteasome subunit beta type 3	IPI00028004	88
	antioxidant	Glutathione S-Transferase	IPI00219757	34
	antioxidant	Peroxiredoxin-2 [9]	IPI00027350	31
	mitochondrial maintenance	Prohibitin-2 [22]	IPI00027252	59
	modulates oxidative stress	Protein DJ-1	IPI00298547	40
10nM	chaperone/foldase	HSP90-beta [5,14]	IPI00414676	241
	chaperone/foldase	HSP90AA1 isoform 1 [5,14]	IPI00784295	77
	chaperone/foldase	HSP90AB4P [5,14]	IPI00555565	49
	chaperone/foldase	BiP	IPI00003362	97
	chaperone/foldase	HSP70 protein 6 [25]	IPI00339269	45
	chaperone/foldase	HSC71 isoform 1[25]	IPI00003865	130
	chaperone/foldase	GRP75	IPI00007765	53
	chaperone/foldase	HSP60, mitochondrial	IPI00784154	29
	chaperone/foldase	HSP90 co-chaperone Cdc37	IPI00013122	70
	chaperone/foldase	HSP70/HSP90 organizing protein [4]	IPI00013894	29
	chaperone/foldase	TCP1 T-complex protein 1 subunit beta [38]	IPI00297779	116
	chaperone/foldase	Peptidyl-prolyl cis-trans isomerase A [36]	IPI00419585	44

Rotenone 48 hrs	Function	Protein	Accession Numbers	Mascot Score
	chaperone/foldase	Protein disulfide-isomerase A6 isoform 2 [36]	IPI00299571	89
	chaperone/foldase	Protein disulfide-isomerase A3 [36]	IPI00025252	38
	chaperone/foldase	Prefoldin subunit 6	IPI00005657	46
	UPS system	Ubiquitin carboxyl-terminal hydrolase isozyme	IPI00018352	51
	UPS system	Ubiquitin-like modifier-activating enzyme 1	IPI00645078	46
	UPS system	Ubiquitin-conjugating enzyme E2	IPI00003949	57
	UPS system	26S proteasome subunit S10B protease	IPI0021926	43
	UPS system	26S proteasome subunit B5 isoform 3	IPI00383971	29
	antioxidant	Glutathione S-transferase	IPI00219757	152
	antioxidant	Superoxide dismutase	IPI00218733	58
	modulates oxidative stress	Protein DJ-1	IPI00298547	62
20nM	chaperone/foldase	HSP90-beta [5,14]	IPI00414676	347
	chaperone/foldase	HSP90-alpha isoform 1 [5,14]	IPI00784295	107
	chaperone/foldase	HSP90B1 endoplasmin/GRP94 [5,14]	IPI00027230	73
	chaperone/foldase	BiP	IPI00003362	77
	chaperone/foldase	HSC71 isoform 1 [25]	IPI00003865	329
	chaperone/foldase	HSP70 protein 7 [25]	IPI00011134	109
	chaperone/foldase	GRP75	IPI00007765	58
	chaperone/foldase	HSP60, mitochondrial	IPI00784154	258
	chaperone/foldase	HSP27 [24,28]	IPI00025512	31
	chaperone/foldase	TCP1 T-complex subunit 3 isoform b [38]	IPI00290770	63
	chaperone/foldase	TCP1 T-complex protein 1 subunit alpha [38]	IPI00290566	91
	chaperone/foldase	TCP1 T-complex protein 1 subunit beta [38]	IPI00297779	52
	chaperone/foldase	TCP1 T-complex protein 1 subunit delta [38]	IPI00302927	72
	chaperone/foldase	TCP1 T-complex protein 1 subunit epsilon [38]	IPI00010720	110
	chaperone/foldase	Peptidyl-prolyl cis-trans isomerase A	IPI00419585	109
	chaperone/foldase	Peptidyl-prolyl cis-trans isomerase B	IPI00646304	60
	chaperone/foldase	Protein disulfide-isomerase A6 isoform 2 [36]	IPI00299571	73
	chaperone/foldase	Protein disulfide-isomerase A3 [36]	IPI00025252	70
	chaperone/foldase	Calreticulin	IPI00020599	38
	UPS system	Ubiquitin-conjugating enzyme E2	IPI00003949	69
	UPS system	Proteasome subunit alpha type-1	IPI00016832	49
	UPS system	Proteasome subunit alpha type-7	IPI00024175	40
	antioxidant	Glutathione S-transferase	IPI00246975	41
	antioxidant	Superoxide dismutase	IPI00218733	92
	antioxidant	Peroxiredoxin-1 [9]	IPI00000874	48
	antioxidant	Peroxiredoxin-6 [9]	IPI00220301	122
	mitochondrial maintenance	Prohibitin-1 [22]	IPI00017334	59
	mitochondrial maintenance	Prohibitin-2 [22]	IPI00027252	78

Rotenone 48 hrs	Function	Protein	Accession Numbers	Mascot Score
	microtubule remodeling	Stathmin	IP00479997	104
	microtubule remodeling	Stathmin-2	IPI00218667	49
	microtubule remodeling	Stathmin-4 isoform 1	IPI00006575	41
	export misfolded proteins	Transitional ER ATPase	IPI00022774	38
	antinflammatory	Cytosolic Phosolipase A2	IPI00384577	54
50nM	chaperone/foldase	HSP90-beta [5,14]	IPI00414676	266
	chaperone/foldase	HSP90-alpha isoform 2 [5,14]	IPI00382470	173
	chaperone/foldase	HSP90B1 endoplasmin/GRP94 [5,14]	IPI00027230	32
	chaperone/foldase	BiP	IPI00003362	71
	chaperone/foldase	HSP75, mitochondrial	IPI00030275	77
	chaperone/foldase	HSP70 protein 1 [25]	IPI00304925	30
	chaperone/foldase	HSP70 protein 6 [25]	IPI00339269	23
	chaperone/foldase	HSC71 isoform 1 [25]	IPI00003865	103
	chaperone/foldase	HSP60, mitochondrial	IPI00784154	182
	chaperone/foldase	HSP10, mitochondrial [11]	IPI00220362	78
	chaperone/foldase	TCP1 T-complex protein 1 subunit alpha [38]	IPI00290566	45
	chaperone/foldase	TCP1 T-complex protein 1 subunit 3 isoform c [38]	IPI00552715	54
	chaperone/foldase	TCP1 T-complex protein 1 subunit zeta [38]	IPI00027626	34
	chaperone/foldase	Peptidyl-prolyl cis-trans isomerase A	IPI00419585	36
	chaperone/foldase	Peptidyl-prolyl cis-trans isomerase B	IPI00646304	52
	chaperone/foldase	ERp57 [7]	IPI0025252	51
	chaperone/foldase	Protein disulfide isomerase A5 [36]	IPI0031479	51
	UPS system	Ubiquitin carboxyl-terminal hydrolase isozyme L1	IPI00018352	62
	UPS system	E3 Ubiquitin ligase UBR5	IPI00026320	35
	UPS system	E3 Ubiquitin ligase MARCH6	IPI00105518	29
	UPS system	Proteasome subunit alpha type-5	IPI00291922	23
	antioxidant	Glutathione S-transferase	IPI00219757	60
	antioxidant	Thioredoxin	IPI00216298	71
	mitochondrial maintenance	Prohibitin-1 [22]	IPI00017334	38
	mitochondrial maintenance	Prohibitin-2 [22]	IPI00027252	54
	microtubule remodeling	Stathmin	IP00479997	37
	neurite growth	Neuron Navigator 2 isoform 1	IPI00217052	27
	antinflammatory	Cytosolic Phosolipase A2	IPI00384577	62
	mitochondrial apoptosis	Porin/VDAC1	IPI00216308	48
	mitochondrial apoptosis	Porin/VDAC2	IPI00241145	54
100nM	chaperone/foldase	HSP90-beta [5,14]	IPI00414676	170
	chaperone/foldase	HSP90-alpha isoform 2 [5,14]	IPI00382470	52
	chaperone/foldase	HSP90AB2P [5,14]	IPI00455599	49

Rotenone 48 hrs	Function	Protein	Accession Numbers	Mascot Score
	chaperone/foldase	HSP90B1 endoplasmin/GRP94 [5,14]	IPI00027230	59
	chaperone/foldase	HSP90 ATPase activator [5,14]	IPI00030706	53
	chaperone/foldase	BiP	IPI00003362	101
	chaperone/foldase	HSP70 protein 1 [25]	IPI00304925	93
	chaperone/foldase	HSP70 protein 4 [25]	IPI00002966	53
	chaperone/foldase	HSP70 protein 6 [25]	IPI00339269	93
	chaperone/foldase	HSC71 isoform 1 [25]	IPI00003865	236
	chaperone/foldase	GRP75	IPI00007765	76
	chaperone/foldase	HSP60	IPI00917575	235
	chaperone/foldase	HSP70/HSP90 organizing protein [3]	IPI00013894	33
	chaperone/foldase	TCP1 T-complex protein 1 subunit beta [38]	IPI00297779	94
	chaperone/foldase	TCP1 T-complex protein 1 subunit delta [38]	IPI00302927	67
	chaperone/foldase	TCP1 T-complex protein 1 subunit epsilon [38]	IPI00010720	109
	chaperone/foldase	Peptidyl-prolyl cis-trans isomerase B	IPI00646304	72
	chaperone/foldase	Protein disulfide-isomerase A3 [36]	IPI00025252	59
	chaperone/foldase	Protein disulfide-isomerase A6 isoform 2 [36]	IPI00299571	107
	chaperone/foldase	Calnexin	IPI00020984	52
	UPS system	Ubiquitin carboxyl-terminal hydrolase isozyme L1	IPI00018352	56
	UPS system	Ubiquitin-like modifier-activating enzyme 1	IPI00645078	38
	UPS system	Proteasome subunit beta type-1	IPI00025019	30
	UPS system	Proteasome subunit beta type-6	IPI00000811	44
	antioxidant	Glutathione S-transferase	IPI00219757	80
	antioxidant	Thioredoxin	IPI00216298	57
	microtubule remodeling	Stathmin	IP00479997	77
	microtubule remodeling	Stathmin-2	IPI00218667	65
	mitochondrial maintenance	Prohibitin-1 [22]	IPI00017334	60
	mitochondrial maintenance	Prohibitin-2 [22]	IPI00027252	70
	export misfolded proteins	Transitional ER ATPase	IPI00022774	46
	suppress apoptosis	Apoptosis Inhibitor 5 isoform 2	IPI00554742	32
	pro-apoptosis	Cytochrome c	IPI00465315	34