Turkish Journal of Molecular Biology & Biotechnology (2016) 1: 11-20

ORIGINAL ARTICLE

Turkish JMBB

Parkin induced cell death upon mitochondrial depolarization is cell type dependent

Gürler AKPINAR, Aylin KANLI, Murat KASAP

Department of Medical Biology/DEKART Proteomics Unit, Kocaeli University Medical School, Kocaeli, Turkey

Keywords
Parkinson's Disease, Parkin, Mitophagy, CCCP, SH-SY5Y

Received: 29 May 2015
Accepted: 28 August 2015

ABSTRACT • Background and aims: Parkin is a ubiquitin-protein ligase that is mutated in autosomal-juvenile Parkinsonism. Parkin's interaction with depolarized mitochondria promotes mitophagy and under normal conditions removal of damaged mitochondria has a protective effect on cell survival. However, if the extent of mitochondrial depolarization is overwhelming, then Parkin helps occurrence of large scale mitochondrial depletion which results in cell death. In this study, the consequences of over expressing wild type Parkin in carbonyl cyanide m-chlorophenylhydrazone (CCCP)-treated SH-SY5Y and HeLa cells were investigated. **Materials and methods:** For this purpose, cell lines expressing TetR protein was established. A wild type Parkin expression cassette that could be induced following tetracycline treatment was introduced to these cells. A HeLa-Parkin cell line that was previously established was also used. **Results:** The results demonstrated that HeLa cells responded to CCCP treatment like HEK293 cells. Twenty five percent cell death occurred within 24 hr in the absence of Parkin while 80% cell death occurred in the presence of Parkin. In contrast, treatment of uninduced SH-SY5Y cells with CCCP caused 60% cell death, and this level was not increased further by Parkin induction. Parkin expression in glucose-depleted medium also rendered HeLa cells but not the SH-SY5Y cells more sensitive to CCCP-induced cell death. **Conclusion:** Finally, Parkin expression protected SH-SY5Y cells from cell death induced by tunicamycin treatment, which induces ER stress by blocking N-linked glycosylation, suggesting that Parkin protects these cells from this type of stress.

INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer (1). PD produces a clinical picture with motor symptoms e.g., tremor, bradykinesia, rigidity, impairment of postural reflexes and postural instability (2) and non motor symptoms e.g., depression, apathy, sleep disorders and erectile dysfunction (3,4). If not treated properly, PD can lower the life quality to an extent that patients cannot carry out their daily activities (5,6). Unfortunately, treatment of PD is symptomatic and current therapies cannot completely eradicate the disease. Although there is a substantial amount of research carried out to find a cure, success is limited mostly because PD is a complex disease with many players involved (7,8). So far, genetic studies identified at least six

Correspondence: Murat Kasap, Kocaeli University Medical School, Department of Medical Biology/DEKART Proteomics Unit, 41380, Umuttepe, Kocaeli, Turkey • Phone: +902623037539 • E-mail: mkasap2008@gmail.com

genes that are well-validated. These include SNCA, LRRK2, PARK2, PINK1, DJ-1, and ATP13A2 (9).

Among these genes we focused on PARK2 which encodes Parkin protein (10). Parkin is an E3 ubiquitin-protein ligase and plays a pivotal role in cell sanitation by helping the removal of unwanted proteins by targeting them to proteosome (11). Although mutations on Parkin are frequently associated with autosomal recessive juvenile or early-onset PD (ARJP or EOPD) Parkinsonism (10,12, 13), evidence suggests that Parkin also plays other roles in cell metabolism and can have a role in the emergence of other diseases such as cancers (2,14).

Mechanism of Parkin's involvement in PD has long been investigated. It is currently thought that the loss of Parkin's targeting function to the proteosome due to disease causing mutations leads to abnormal accumulation of toxic proteins and neurodegeneration (15). Recent studies, however, accumulated multiple lines of evidence from animal models, in vitro and patient-based studies and demonstrated the involvement of Parkin not only in impaired protein turnover but also in mitochondrial dysfunction (16,17). In other words, Parkin also functions as a potent mitochondrial protection factor. Based on the gathered experimental data, a model for the maintenance of mitochondrial integrity by Parkin was proposed (18). In that model, PINK-1 which is a PTEN-induced protein kinase (19) phosphorylates Parkin to translocate it from cytosol to mitochondria where it exerts its mito-protective effects (20). Phosphorylation of Parkin enhances the degradation of dysfunctional mitochondria by targeting them to autophagosomes, and replaces the discarded mitochondria by promoting mitochondrial biogenesis (18, 21). Based on this information, several assumptions can be made: (1) If mitochondria is chemically depolarized in the cell then Parkin should be able to remove the defective mitochondria. (2) If the extent of depolarization is kept high and Parkin is overexpressed, then the overexpressed Parkin should increase the rate of removal of defective mitochondria. The increase in the rate of mitochondrial removal (mitophagy) should reflect onto the rate of cell death.

Morrison et al. (2011) tested these assumptions and proposed a simple cell based assay to measure Parkin's activity (22). In their study, the authors clearly demonstrated that Parkin caused an increase in the rate of removal of CCCP-treated cells. In this study, we used their assay to measure activity of Parkin protein in HeLa and SH-SY5Y cells. Our findings suggested that Parkin induced cell death upon mitochondrial depolarization is cell type dependent. In this study, we also measured Parkin's activity by using an assay which relied on ER-stress created by tunicamycin. This assay appeared to be cell-type independent.

MATERIALS AND METHODS

Expression plasmids

Full-length cDNA of human WT Park2 was cloned into pCDNA4/TO (Life Tech, USA) by reverse transcription and long PCR with appropriate primers (23). The construct was sequenced and in-frame Parkin sequence was verified (Iontek Inc., Turkey). pCDNA6/TR was from Life Technologies (Life Tech, USA).

Cell culture and preparation of stable cell lines expressing WT and mutant Parkin proteins

SH-SY5Y cells were grown in EMEM supplemented with 10 % (vol/vol) tetracycline-reduced fetal bovine serum, 100 U/ml penicillin-streptomycin and 2 mM L-glutamine at 37°C in a humidified 5% CO_2 atmosphere. To prepare Tet-R^+ stable SH-SY5Y cells, pcDNA6/TR was transfected to SH-SY5Y cells by electroporation and the transfected cells were subjected to a selection in a medium containing 5 µg/ml blasticidin. Individual clones were isolated, grew and examined for TetR protein expression by western blotting with an anti-TetR antibody (Clontech, USA). One of the colonies which

highly expressed TetR protein was labelled as SH-SY5Y-TetR$^+$ and used in subsequent experiments.

SH-SY5Y-TetR$^+$ cells were transfected with pcDNA4/TO harboring WT PARK2 to create an inducible stable cell line expressing WT Parkin protein. Transfected cells were then subjected to a selection in the medium containing 50 µg/ml zeocin. Individual clones were isolated, grew and examined for Parkin protein expression by western blotting with an anti-Parkin antibody (Santa Cruz, USA). One of the screened colony expressing WT after tet induction, labeled as SH-SY5Y-TetR$^+$ -WT-Parkin, was selected for subsequent studies.

CCCP-treatment and whole-cell Parkin assay

10^3 cells were plated into each well of a 96-well plate and allowed to attach for 16 hr. Media was then replaced with either standard media or standard media containing 1µg/ml tetracycline. After 16 hr. of induction, 10 µM CCCP was added to the relevant wells and the cells were incubated for 24 additional hours (22). The cells were then subjected to WST-1 assay (Roche Inc., USA). The absorbance of each well was determined at 450 nm with a plate reader (Multiscan FC, Thermo Scientific, USA) and the background values were subtracted. Results were presented as percentages of the controls. Each assay was performed in triplicate and the experiments were repeated at least twice.

CCCP treatment and whole-cell Parkin assay in glucose-free medium

10^3 cells of each cell line were plated into each well of a 96-well plate and allowed to attach for 16 hr. Media was then replaced with either standard media or standard media containing 1µg/ml tetracycline. After 16 hr. of induction, to the relevant wells 10 µM CCCP was added and the cells were incubated for 24 additional hours. The standard media were then washed with cold PBS for three times and replaced with glucose-free galactose supplemented media. The cells were allowed to grow for four days and then were subjected to WST-1 assay (Roche Inc., USA). The absorbance of each well was determined at 450 nm with a plate reader (Multiscan FC, Thermo Scientific, USA) and the background values were subtracted. Results were presented as percentages of the controls. Each assay was performed in triplicate and the experiment was repeated at least twice.

Trypan blue exclusion assay

The number of death cells was determined by trypan-blue exclusion assay (24). 2×10^5 cells of each cell lines were plated into each well of a 24-well plate and allowed to attach for 16 hr. Media was then replaced with either standard media or standard media containing 1µg/ml tetracycline. After 16 hr. of induction, 10 µM CCCP was added to the relevant wells and the cells were incubated for 24 additional hours. Cell suspensions were then simply mixed with trypan blue solution (0.4% w/v trypan blue in 1 × PBS) and then visually examined by an inverted microscope to determine whether cells took up (non-viable) or excluded the dye (viable). Cell counting was performed with a hemocytometer and the percent of viable cells was calculated.

Tunicamycine treatment and whole-cell Parkin assay

The experiment was performed as explained under the title of "CCCP-treatment and whole-cell Parkin assay" except that 10 µM tunicamycine was used instead of 10 µM CCCP.

Real-Time PCR to determine Bip expression levels

Total RNA was isolated from cultured cells by using RNA isolation kit (Qiagen, USA) and the first strand synthesis was performed by using oligo-dT primers (Thermo Scientific, USA). RT-PCR was performed by using RT2-SYBR Green qPCR mix (Qiagen, USA). The primer pair used in RT-PCR reaction were (sense) 5'-CCCAACTGGCTGGCAA-

GATG-3' and (antisense) 5'-TGGAGGTGAGCT-GGTTCTTGG-3'. The results were analyzed with REST 2009 (Qiagen, USA).

RESULTS

Generation of Parkin inducible SH-SY5Y cell line

In this study, we performed inducible Parkin expression experiments by using the Tet-on regulated gene expression system (25). The expression was tight enough that only after the induction, Parkin protein can be detectable on the western blots. When a time dependent expression study was performed, Parkin expression started to appear on blots after 5 hr. of induction when 5 µg total protein was used in mini-gels for western blotting (Figure 1). A previously created HeLa cell line expressing Parkin protein also displayed a tightly controlled Parkin expression (26).

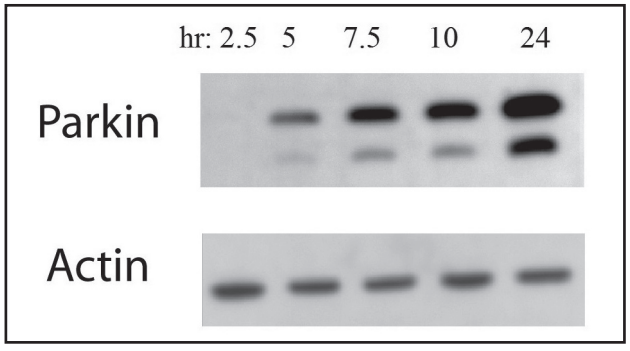

Figure 1 Time-dependent expression of Parkin protein after induction in SH-SY5Y cells.

Whole cell Parkin assay

Morrisson et al. (2011) studied the effect of inducing Parkin expression in inducible HEK-293 cells cultured in the presence of mitochondrial uncoupler CCCP (22). They determined that after 24 hr. of CCCP treatment, approximately 50% of the cells lost their viability. When they expressed Parkin in CCCP treated cells, the number of viable cells decreased dramatically and approximately 90% of the cells died. We repeated the experiments of Morrison et al. (2011) by using our Parkin inducible SH-SY5Y cell line. Sixty three percent of the CCCP-treated SH-SY5Y cells, which lacked expressed Parkin lost their viability within 24 hr. Similarly, 57% of the CCCP-treated and Parkin expressing SH-SY5Y cells lost their viability within 24 hr. (Figure 2A).

To explain the disagreement between our results and the results obtained by Morrision et al. (2011), we included a control experiment to our assay in which we used a Parkin inducible HeLa cell line (Figure 2A). HeLa cells originate from non-neuronal cells like HEK293, whereas SH-SY5Y cells originate from brain and display motor-neuron like properties (27). Twenty six percent of the CCCP-treated HeLa cells which lacked expressed Parkin lost their viability within 24 hr. However, when Parkin expression was induced 80% of the CCCP-treated HeLa cells lost their viability within 24 hr indicating that Parkin expression caused a significant decrease in cell viability. To eliminate the possibility that the differences in cell death response to CCCP treatment are simply a result of different levels of Parkin expression, the levels of Parkin after 24 hr of induction in both HeLa and SH-SY5Y cells were determined. The result demonstrated that cell death response to CCCP treatment was not due to different levels of Parkin expression (Figure 2B).

WST-1 assay used in above experiments to assess cell viability is based on the cleavage of the tetrazolium salt (WST-1) to formazan by cellular mitochondrial dehydrogenases. We predicted that CCCP, which directly affects mitochondrial function through its protonophore action might affect WST-1 assay and thus additional measures of cell viability were needed. We therefore performed tryphan blue exclusion assay to see if the results of WST-1 assay would be similar to the results of tryphan-blue exclusion assay. Although the figures were not exactly the same, the results obtained

from tryphan blue assay also demonstrated that unlike HeLa cells, over expression of Parkin did not cause a change in cell viability in CCCP-treated SH-SY5Y cells (Figure 2C).

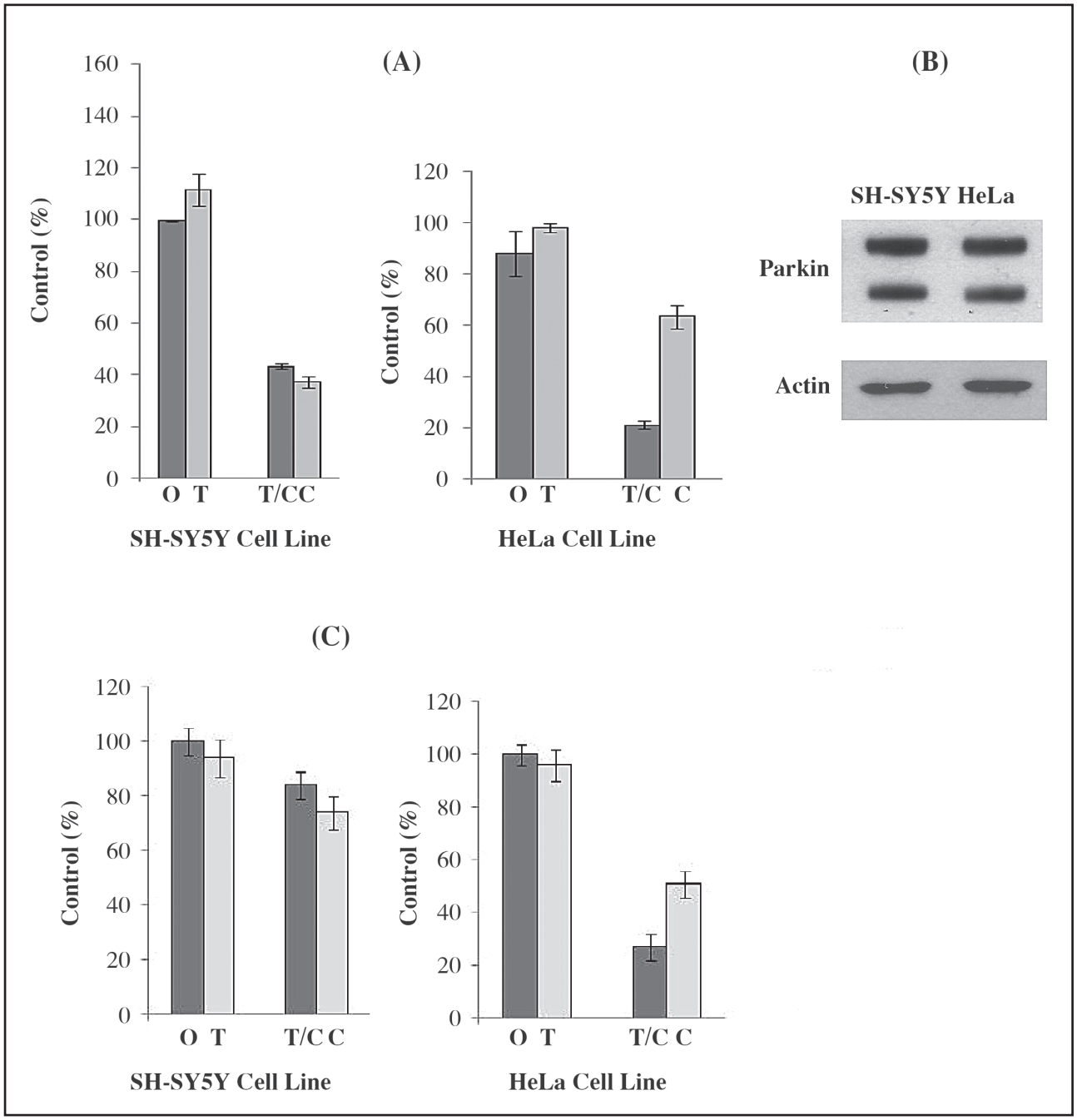

Figure 2 **(A)** WST-1 assay to demonstrate the effect of Parkin expression on cell survival in CCCP-treated cells. **(B)** Parkin expression in SH-SY5Y and HeLa cells after 24 hr of induction. **(C and D)** Tryphan blue exclusion assay to demonstrate the effect of Parkin expression on cell survival in CCCP-treated cells. Letters represent: (O) control culture in standard media, (T) culture in standard media supplemented with tetracycline for Parkin induction, **(C)** culture in standard media supplemented with CCCP, (T/C) culture in standard media supplemented with tetracycline and CCCP. Statistical comparisons were carried out between groups T and T/C by using independent 2-sample *t*-test ($P < 0.01$).

Biochemical evidence for SH-SY5Y cell survival in CCCP-supplemented media

Cells grown in glucose media generate their ATP by glycolysis largely bypassing the mitochondria. However, when cells are grown in galactose supplemented media, they are forced to use oxidative phosphorylation for generation of ATP. When mitochondria are damaged in galactose supplemented glucose-free media, the cells then become more vulnerable to mitochondrial dysfunction. Therefore, by growing up a group of cells in galactose supplemented media, the extent of mitochondrial damage can be measured (28). To provide biochemical evidence that SH-SY5Y cells expressing Parkin preserves mitochondrial function after CCCP-treatment, we carried out an experiment in glucose-free galactose supplemented medium. Approximately 70% of the HeLa cells expressing Parkin protein in galactose medium lost their viability (Figure 3A). On the other hand, under the same culture conditions, in CCCP-treated SH-SY5Y cells a high percentage of live cells were encountered after CCCP-treatment (Figure 3B). The findings of this experiment were similar to the findings of the cell-based Parkin assay.

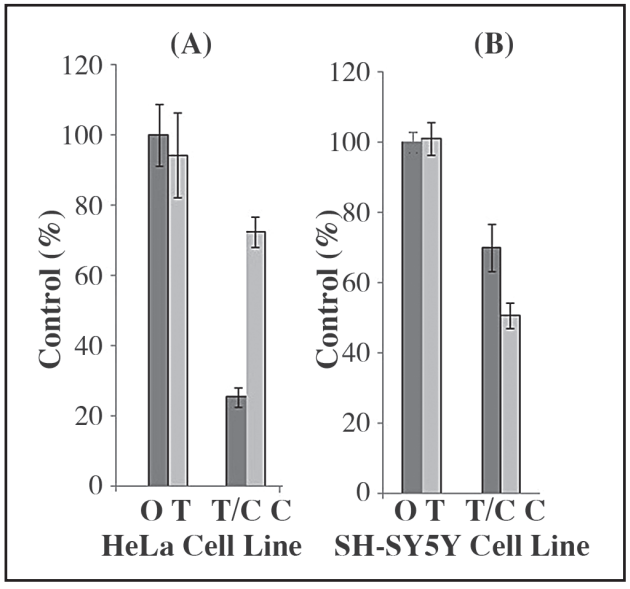

Figure 3 WST-1 assay in galactose supplemented glucose deficient media. Letters represent: (O) control culture in galactose supplemented glucose deficient media, (T) culture in galactose supplemented glucose deficient media with tetracycline for Parkin induction, (C) culture in galactose supplemented glucose deficient media supplemented with CCCP, (T/C) culture in galactose supplemented glucose deficient media supplemented with tetracycline and CCCP. Statistical comparisons were carried out between groups T and T/C by using independent 2-sample *t*-test ($P < 0.01$).

ER stress by tunicamycin induction may be a way to assay activity of Parkin

Abnormal accumulation of unfolded proteins in the ER is a constant threat to cell viability and thus cells possess several mechanisms to fight against this stress. However, despite the unfolded protein response, cell death occurs if the amount of accumulated unfolded protein exceeds a threshold level. A previous study demonstrated that Parkin is able to suppress unfolded protein stress and able to prevent cell death through its E3 ubiquitin protein ligase activity (19). In a recent study, similar findings were also reported by Bouman et al. (2011) who transiently expressed the wild type and the mutant Parkin proteins in SH-SY5Y cells treated with tunicamycin and analyzed cells with caspase-3 antibody using immunofluorescence microscopy (29). In this study, we used a simple MTT-based assay (WST-1) for measuring the cell-based Parkin activity. Cells treated with tunicamycin went under stress as demonstrated by an increase in the Bip mRNA level (12-fold increase upon treatment) (Figure 4A). When tunicamycin-treated cells were assayed with a modified MTT reagent in 96-well plates to assess their viabilities, we observed that 33% of the SH-SY5Y cells treated with tunicamycin in the absence of over expressed WT Parkin lost their viability, while only 15 % of the SH-SY5Y cells treated with tunicamycin in the presence of over expressed Parkin lost their viability (Figure 4B). Similar results were also obtained with HeLa cells indicating that irrespective of the cell type Parkin helped to preserve cellular function and cell viability under stress and this property of Parkin may be used to assess its biologic activity.

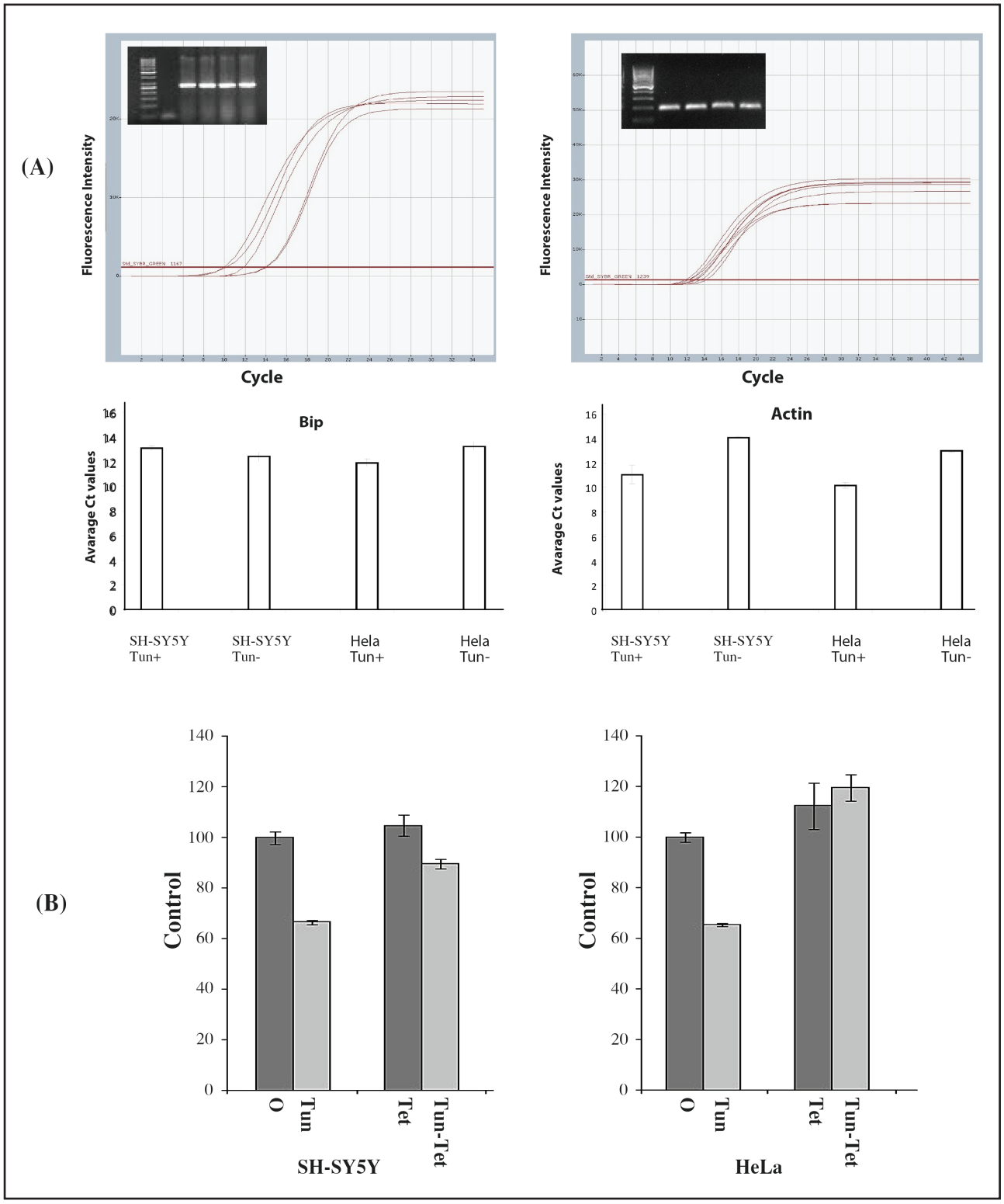

Figure 4 **(A)** RT-PCR analysis to demonstrate the increase in Bip mRNA level upon tunicamycine induced cell stress. **(B)** WST-1 assay after Parkin induction in tunicamycine treated cells. Letter (O) represent the control culture in standard media. Statistical comparisons were carried out between groups Tun and Tun-Tet by using independent 2-sample *t*-test ($P < 0.01$).

DISCUSSION

Cells can selectively target their impaired mitochondria to autophagosomes for removal and Parkin plays a pivotal role in this targeting process. Evidence for Parkin's recruitment on impaired mitochondria was first recognized in HEK-293 cells (30). The findings have led to an emerging model where mitochondrial dysfunction was placed into the center of PD. Follow up studies tried answering several important questions about the removal of damaged mitochondria, including how Parkin is recruited to impaired mitochondria, what other proteins play roles in this process, what marks the dysfunctional mitochondria and how Parkin distinguishes dysfunctional mitochondria from the functional ones (31-37). The reported published findings, in overall, indicate that (a) Parkin localizes to and ubiquitinylates mitochondria with low membrane potential. (b) Disease-relevant mutations of Parkin impair mitochondrial localization. (c) Parkin exerts its E3 activity only when the mitochondrial membrane potential decreases. (d) PINK1 retrieves Parkin from the cytosol to the mitochondria. (e) PINK1 localization is stabilized by damaged mitochondria. (f) Mitochondrial localization and kinase activity of PINK1 is essential for translocation of Parkin to damaged mitochondria.

It is still not clear if Parkin's recruitment is a general response to mitochondrial stress. Also, to what extent Parkin mediates mitochondrial fidelity is not known. Is there a possibility that different cell types may respond differently to mitochondrial insult? Are there differences in mitochondrial removal process or is there a universal mechanism that uses the same or similar players? What alternative factors may promote mitophagy in different cell types? Certain cell types such as neurons or brain dwelled cells may require much different intracellular mitochondrial surveillance than proliferating cell populations. In this respect, Van Laar et al., (2011) investigated Parkin/PINK1 dependent mitophagy phenomenon in primary cortical neurons isolated from rats and found that in neurons unlike other cell types, CCCP did not induce Parkin translocation to mitochondria or mitophagy and there was no correlation between mitochondrial depolarization with CCCP and Parkin-mitochondrial localization (38). What they pointed out in their study was that much of the Parkin-mediated mitophagy pathway had been investigated in immortalized cell lines and these cell lines may respond to CCCP mediated mitochondrial insult differently than primary neurons. Our observations in this study showed that brain-derived SH-SY5Y cells displayed a different response to CCCP-treatment and Parkin induced cell death upon mitochondrial depolarization was cell type dependent.

The tunicamycin based assay, we proposed here, may be more universal then MTT-based assay because the MTT-based assay partly relies on mitochondrial reductase enzyme activity and thus are dependent on the presence of metabolically active mitochondria. Since CCCP may directly affect mitochondrial function through its protonophore action, it may be better to use an assay method that does not rely on mitochondrial activity. In this respect, tunicamycin-based assay is a good candidate because the assay does not have any side effect on mitochondrial activity.

REFERENCES

1. Alves G, Forsaa EB, Pedersen KF, Dreetz Gjerstad M, Larsen JP. Epidemiology of Parkinson's disease. J Neurol. 2008 Sep;255 Suppl 5:18-32.
2. Shahed J, Jankovic J. Motor symptoms in Parkinson's disease. Handb Clin Neurol. 2007;83:329-42.
3. Chaudhuri KR, Schapira AH. Non-motor symptoms of Parkinson's disease: dopaminergic pathophysiology and treatment. Lancet Neurol. 2009 May;8(5):464-74.
4. Kagi G, Klein C, Wood NW, Schneider SA, Pramstaller PP, Tadic V, et al. Nonmotor symptoms in Parkin gene-related parkinsonism. Mov Disord. 2010 Jul 15;25(9):1279-84.

5. Uitti RJ. Treatment of Parkinson's disease: focus on quality of life issues. Parkinsonism Relat Disord. 2011;18 Suppl 1:S34-6.
6. Shulman LM, Gruber-Baldini AL, Anderson KE, Vaughan CG, Reich SG, Fishman PS, et al. The evolution of disability in Parkinson disease. Mov Disord. 2008 Apr 30;23(6):790-6.
7. Klein C, Schlossmacher MG. Parkinson disease, 10 years after its genetic revolution: multiple clues to a complex disorder. Neurology. 2007 Nov 27;69(22):2093-104.
8. Giroux ML. Parkinson disease: managing a complex, progressive disease at all stages. Cleve Clin J Med. 2007 May;74(5):313-4, 7-8, 20-2 passim.
9. Kumar KR, Djarmati-Westenberger A, Grunewald A. Genetics of Parkinson's disease. Semin Neurol. 2012 2012;31(5):433-40.
10. Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Minoshima S, et al. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. Nature. 1998 Apr 9;392(6676):605-8.
11. Rankin CA, Roy A, Zhang Y, Richter M. Parkin, A Top Level Manager in the Cell's Sanitation Department. Open Biochem J. 2011;5:9-26.
12. Shimizu N, Asakawa S, Minoshima S, Kitada T, Hattori N, Matsumine H, et al. PARKIN as a pathogenic gene for autosomal recessive juvenile parkinsonism. J Neural Transm Suppl. 2000(58):19-30.
13. Sironi F, Primignani P, Zini M, Tunesi S, Ruffmann C, Ricca S, et al. Parkin analysis in early onset Parkinson's disease. Parkinsonism Relat Disord. 2008;14(4):326-33.
14. Picchio MC, Martin ES, Cesari R, Calin GA, Yendamuri S, Kuroki T, et al. Alterations of the tumor suppressor gene Parkin in non-small cell lung cancer. Clin Cancer Res. 2004 Apr 15;10(8):2720-4.
15. Wakabayashi K, Mori F, Tanji K, Orimo S, Takahashi H. Involvement of the peripheral nervous system in synucleinopathies, tauopathies and other neurodegenerative proteinopathies of the brain. Acta Neuropathol. 2010 Jul;120(1):1-12.
16. Darios F, Corti O, Lucking CB, Hampe C, Muriel MP, Abbas N, et al. Parkin prevents mitochondrial swelling and cytochrome c release in mitochondria-dependent cell death. Hum Mol Genet. 2003 Mar 1;12(5):517-26.
17. Kuroda Y, Mitsui T, Kunishige M, Matsumoto T. Parkin affects mitochondrial function and apoptosis in neuronal and myogenic cells. Biochem Biophys Res Commun. 2006 Sep 29;348(3):787-93.
18. Bueler H. Impaired mitochondrial dynamics and function in the pathogenesis of Parkinson's disease. Exp Neurol. 2009 Aug;218(2):235-46.
19. Heutink P. PINK-1 and DJ-1–new genes for autosomal recessive Parkinson's disease. J Neural Transm Suppl. 2006(70):215-9.
20. Kim Y, Park J, Kim S, Song S, Kwon SK, Lee SH, et al. PINK1 controls mitochondrial localization of Parkin through direct phosphorylation. Biochem Biophys Res Commun. 2008 Dec 19;377(3):975-80.
21. de Pablo-Latorre R, Saide A, Polishhuck EV, Nusco E, Fraldi A, Ballabio A. Impaired parkin-mediated mitochondrial targeting to autophagosomes differentially contributes to tissue pathology in lysosomal storage diseases. Hum Mol Genet. 2012 Apr 15;21(8):1770-81.
22. Morrison E, Thompson J, Williamson SJ, Cheetham ME, Robinson PA. A simple cell based assay to measure Parkin activity. J Neurochem. 2011 Feb;116(3):342-9.
23. Kasap M, Akpinar G, Sazci A, Idrisoglu HA, Vahaboglu H. Evidence for the presence of full-length PARK2 mRNA and Parkin protein in human blood. Neurosci Lett. 2009 Sep 4;460(3):196-200.
24. Strober W. Trypan blue exclusion test of cell viability. Curr Protoc Immunol. 2001 May;Appendix 3:Appendix 3B.
25. Ozgul S, Kasap M, Akpinar G, Kanli A, Guzel N, Karaosmanoglu K, et al. Linking a compound-heterozygous Parkin mutant (Q311R and A371T) to Parkinson's disease by using proteomic and molecular approaches. Neurochem Int. Jun-Jul;85-86:1-13.
26. Kasap M, Thomas S, Danaher E, Holton V, Jiang S, Storrie B. Dynamic nucleation of Golgi apparatus assembly from the endoplasmic reticulum in interphase hela cells. Traffic. 2004 Aug;5(8):595-605.
27. Xie HR, Hu LS, Li GY. SH-SY5Y human neuroblastoma cell line: in vitro cell model of dopaminergic neurons in Parkinson's disease. Chin Med J (Engl). 2010 Apr 20;123(8):1086-92.
28. Swiss R, Will Y. Assessment of mitochondrial toxicity in HepG2 cells cultured in high-glucose- or galactose-containing media. Curr Protoc Toxicol. 2011 Aug;Chapter 2:Unit2 20.
29. Bouman L, Schlierf A, Lutz AK, Shan J, Deinlein A, Kast J, et al. Parkin is transcriptionally regulated by ATF4: evidence for an interconnection between mitochondrial stress and ER stress. Cell Death Differ. 2010 May;18(5):769-82.
30. Narendra D, Tanaka A, Suen DF, Youle RJ. Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. J Cell Biol. 2008 Dec 1;183(5):795-803.
31. Lee JY, Nagano Y, Taylor JP, Lim KL, Yao TP. Disease-causing mutations in parkin impair mitochondrial ubiquitination, aggregation, and HDAC6-dependent mitophagy. J Cell Biol. 2010 May 17;189(4):671-9.
32. Matsuda N, Sato S, Shiba K, Okatsu K, Saisho K, Gautier CA, et al. PINK1 stabilized by mitochondrial depolarization recruits Parkin to damaged mitochondria and activates latent Parkin for mitophagy. J Cell Biol. 2010 Apr 19;189(2):211-21.
33. Narendra D, Tanaka A, Suen DF, Youle RJ. Parkin-induced mitophagy in the pathogenesis of Parkinson disease. Autophagy. 2009 Jul;5(5):706-8.
34. Narendra DP, Jin SM, Tanaka A, Suen DF, Gautier CA, Shen J, et al. PINK1 is selectively stabilized on impaired mitochondria to activate Parkin. PLoS Biol. 2010 Jan;8(1):e1000298.
35. Poole AC, Thomas RE, Andrews LA, McBride HM, Whitworth AJ, Pallanck LJ. The PINK1/Parkin pathway regulates mitochondrial morphology. Proc Natl Acad Sci U S A. 2008 Feb 5;105(5):1638-43.

36. Tanaka A, Cleland MM, Xu S, Narendra DP, Suen DF, Karbowski M, et al. Proteasome and p97 mediate mitophagy and degradation of mitofusins induced by Parkin. J Cell Biol. 2010 Dec 27;191(7):1367-80.

37. Whitworth AJ, Pallanck LJ. The PINK1/Parkin pathway: a mitochondrial quality control system? J Bioenerg Biomembr. 2009 Dec;41(6):499-503.

38. Van Laar VS, Arnold B, Cassady SJ, Chu CT, Burton EA, Berman SB. Bioenergetics of neurons inhibit the translocation response of Parkin following rapid mitochondrial depolarization. Hum Mol Genet. 2011 Mar 1;20(5):927-40.