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Mitochondrial Remodeling Following Fission Inhibition by 15d-PGJ2 Involves Molecular Changes in Mitochondrial Fusion Protein OPA1

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Abstract

We showed earlier that 15 deoxy $\Delta^{12,14}$ prostaglandin J2 (15d-PGJ2) inactivates Drp1 and induces mitochondrial fusion [1]. However, prolonged incubation of cells with 15d-PGJ2 resulted in remodeling of fused mitochondria into large swollen mitochondria with irregular cristae structure. While initial fusion of mitochondria by 15d-PGJ2 required the presence of both outer (Mfn1 and Mfn2) and inner (OPA1) mitochondrial membrane fusion proteins, later mitochondrial changes involved increased degradation of the fusion protein OPA1 and ubiquitination of newly synthesized OPA1 along with decreased expression of Mfn1 and Mfn2, which likely contributed to the loss of tubular rigidity, disorganization of cristae, and formation of large swollen degenerated dysfunctional mitochondria. Similar to inhibition of Drp1 by 15d-PGJ2, decreased expression of fission protein Drp1 by siRNA also resulted in the loss of fusion proteins. Prevention of 15d-PGJ2 induced mitochondrial elongation by thiol antioxidants prevented not only loss of OPA1 isoforms but also its ubiquitination. These findings provide novel insights into unforeseen complexity of molecular events that modulate mitochondrial plasticity.

INTRODUCTION

Dynamic changes in mitochondrial shape or morphology, crucial for normal cellular homeostasis, are controlled by a balance of two opposing processes, fission and fusion [2,3]. While mitochondrial fission is directed by a cytoplasmic GTPase protein, dynamin related protein 1 (Drp1; [4]) and Fis1 [5], mitochondrial fusion is executed by mitochondrial GTPases, mitofusin-1 and -2 (Mfn1 and Mfn2), on the outer membrane [6], and the inner membrane OPA1 [3,7,8]. Disruption of either fusion process due to loss of mitofusins or OPA1 [9] or fission process due to loss of Drp1 have been shown to result in cellular dysfunction, reduced respiration, loss of mitochondrial membrane potential and growth inhibition [9,10]. We recently reported that 15d-PGJ2, a cyclopentenone prostaglandin induced mitochondrial fusion through inhibition of GTPase activity of mitochondrial fission protein Drp1[1]. In view of recent reports that loss of mitochondrial fission by RNAi mediated loss of Drp1 resulted in mitochondrial dysfunction through loss of mitochondrial DNA, decreased mitochondrial respiratory activity and ATP levels [11], we asked the

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question how prolonged inhibition of Drp1 activity would affect the mitochondrial structure and function. In this study, we analyzed the effects of 15d-PGJ2 over time and observed mitochondrial remodeling through formation of degenerated swollen mitochondria with irregular cristae structures in the mitochondrial network due to reduction in fusion protein levels and increased proteolytic processing of OPA1.

MATERIALS AND METHODS

Materials

Rat kidney proximal tubule cells (RPTC) were cultured in Ham's F-12/DMEM supplemented with serum. Mouse embryonic fibroblasts (MEF) (WT, Mfn1-Null, Mfn2-Null, Mfn1/2 double knockout and OPA1-null cells, a kind gift from Dr. David Chan) were grown in DMEM supplemented with serum. N-acetyl cysteine (NAC) was purchased from Calbiochem (San Diego, CA). qVD-OPH from MP Biomedicals (Aurora, OH). N-2-mercapto-propionyl glycine (NMPG) were obtained from Sigma Aldrich (St Louis, MO). 15-deoxy- $\Delta^{12,14}$ Prostaglandin J2 was from Cayman chemicals (Ann Arbor, MI). Ubiquitin antibody was from Biomol (Plymouth Meeting, PA). GAPDH antibody was from Research Diagnostic, INC. (Flanders, NJ). Drp1 and OPA1 antibodies are from BD Biosciences. Fis1 antibody was from Alexis Biochemicals (Lausen, Switzerland). Mfn2 antibody was a kind gift from Dr. Richard Youle and from Proteintech Group, Inc. (Chicago, IL). Mfn1 antibody was from Lifespan Biosciences (Seattle, WA). Horseradish peroxidase-conjugated and pre-adsorbed secondary antibodies to mouse and rabbit were obtained from Jackson ImmunoResearch Laboratories (Westgrove, PA). Mitochondria-specific dye MitoTracker Red was from Invitrogen (Carlsbad, CA) and plasmid expressing MitoDsRed was from Clontech (Mountain View, CA). All other reagents were of the highest grade available.

15d-PGJ2 treatment

Rat kidney proximal tubule cells (RPTC) were treated with 15d-PGJ2 as has been described earlier [1]. Briefly, after overnight growth, cells were incubated with 20 μ M of 15d-PGJ2 or DMSO in fresh growth medium for indicated times. NAC and NMPG were incubated with cells for at least 1 hr in full growth media before 15d-PGJ2 treatment.

Electron microscopy

Electron microscopy was performed as described before [1]. Briefly, control and 15d-PGJ2 treated cells were washed and fixed with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer pH 7.4. Fixed cells were incubated with 1% osmium tetroxide (OsO_4) for 1 hr at 4°C and after 20 min incubation at 4°C in 1% uranyl acetate; cells were washed thoroughly and further processed for electron microscopy.

Western blotting

BCA reagent from Pierce was used to estimate protein concentration and proteins were resolved by reducing SDS-PAGE in Xcell II mini cell on 3–8%, or 4–12% (gradient) NuPAGE gels (Invitrogen, CA) using MES, MOPS or Tris-acetate running buffer as recommended by the manufacturer. After electrophoresis, proteins were electroblotted onto 0.2 μ m PVDF membranes and were analyzed by western blotting using appropriate primary antibodies and peroxidase-conjugated suitable secondary antibodies. Antigen-antibody complexes on PVDF membrane were detected by chemiluminescent substrates from Pierce Biotechnology (Rockford, IL).

Mitochondrial morphology

Mitochondrial morphology was observed as has been described earlier [1]. Briefly, cells were grown on coverslip and after treatment with 15d-PGJ2 or other drugs for the indicated hours, MitoDsred-RPTC or RPTCs stained with mitotracker red, were mounted on grooved chambered slides with dye-free medium and were observed in Olympus FV-1000 Laser Scanning Confocal Microscope. To assess the changes in mitochondrial morphology, pictures were taken in a blinded fashion and at least 100 cells were counted for each time point in three independent experiments.

Measurement of ATP

Control and experimental cells were lysed in perchloric acid and ATP in cell extract was measured by the luciferin firefly luciferase reaction using a luminometer [12].

Blue Native PAGE

Homogenization and solubilization of cells and blue native PAGE (BN-PAGE) was done as described earlier [1].

Immunoprecipitation of OPA1

The cells were lysed with 0.1ml of extraction buffer (1% SDS, 50mM Tris-HCl pH 7.5 and 150mM NaCl) containing benzonase to digest nucleic acids. Lysates were cleared by centrifugation and were diluted to 1ml by adding 900 μ l of 1x dilution buffer (50mM Tris-HCl pH 7.5, 150mM NaCl, 1.12% NP40, 1.12% sodium deoxycholate). OPA1 antibody was bound to pre-washed protein G beads. The beads were then blocked with 1mg BSA and washed once with IP buffer (150mM NaCl, 1.5 mM MgCl₂, 1mM EGTA, 20 mM HEPES pH 7.4 buffer containing 1% NP40). The diluted extracts were added to the beads and rotated at 4°C for 2h. The beads were then washed three times with IP buffer and extracted with 20 μ l of 2x sample buffer containing 20 mM DTT and eluates were subjected to SDS-PAGE and blotting as described above. The blots were probed with OPA1 and Ubiquitin antibodies.

Drp1 Knockdown

RPTC cells were transfected with Drp1 shRNA construct in pREP4 [13], a kind gift from Dr. Richard Youle from NINDS/NIH, and stably expressing clones with Drp1 knockdown were selected by hygromycin.

RESULTS

Prolonged inhibition of Drp1 function results in mitochondrial remodeling

Rat kidney proximal tubular cells (RPTC) deficient in mitochondrial fission protein Drp1 contained relatively lower levels of fusion proteins (OPA1, Mfn1 and Mfn2) than the wild type cells (Fig 1A). This prompted us to investigate the effect of cyclopentenone prostaglandin 15d-PGJ2, which we reported to inhibit Drp1 function and convert small and stubby mitochondria into elongated mitochondria within 2h of treatment [1]. Further incubation with 15d-PGJ2 resulted in remodeling of these fused mitochondria into large swollen structures, which are fewer in number than total number of mitochondria in untreated cells (Fig 1B). As shown in Fig. 1C, the number of cells with elongated mitochondria increased from <10% in untreated cells to ~ 90% within 2h of 15d-PGJ2 treatment. However, by 6h of treatment, >90% of the cells contained large swollen mitochondria.

15d-PGJ2-induced mitochondrial elongations require both outer and inner membrane fusion proteins

We asked the question whether 15d-PGJ2 induced elongations of mitochondria require fusion activity of one or all of the fusion proteins. We addressed this by using mouse embryonic fibroblasts (MEFs) that lacked one or more of the fusion proteins (Fig. 2A). Wild type MEFs contain relatively longer mitochondria than RPTC and 15d-PGJ2 treatment caused further elongation and entanglement of their mitochondria (Fig. 2B). In contrast to wild type MEFs, untreated Mfn1/2-double null or OPA1-null MEFs had punctiform mitochondria which failed to undergo elongation with 15d-PGJ2 treatment (Fig. 2B), suggesting that complete lack of fusion activity in either outer or inner membrane would prevent 15d-PGJ2 mediated mitochondrial elongation. Lack of either one of the mitofusin isoforms did not prevent 15d-PGJ2-induced mitochondrial fusion (Fig. 2C).

Ultrastructural and functional changes in 15d-PGJ2 treated mitochondria

Electron microscopic analysis of 15d-PGJ2 induced changes in mitochondrial morphology revealed that mitochondria were small and round in control cells, 2h treatment with 15d-PGJ2 resulted in mitochondrial elongation (Fig. 3A). However, after 6h of 15d-PGJ2 treatment, long cylindrical shaped mitochondria were replaced by large irregular and swollen mitochondria with disorganized cristae structure (Fig. 3A). To correlate these ultrastructural changes in mitochondria with their function, we measured total cellular ATP levels with or without 15d-PGJ2 treatment in the presence of qVD, which inhibited apoptosis. There were no significant differences in ATP levels between control and treated cells in normal growth medium that contained glucose. However, when cells were forced to use oxidative metabolism for ATP generation in the medium containing pyruvate, glutamate and malate without glucose, ATP levels decreased progressively during 15d-PGJ2 treatment (Fig. 3B). To further assess the quality of mitochondria, mitochondrial fusion protein OPA1 was analyzed by BN-PAGE. OPA1 from control cells migrated as two distinct oligomeric complexes of molecular weight ~ 150 kD and ~ 550 kD (Fig. 3C). 15d-PGJ2 treatment dramatically reduced the levels of oligomeric forms of OPA1 by 6h of treatment (Fig. 3C).

Ubiquitination and proteolysis of OPA1 and loss of mitofusins upon prolonged exposure to 15d-PGJ2

To understand the time dependent changes in mitochondrial morphology and loss of OPA1 complexes by 15d-PGJ2, fusion and fission proteins were analyzed by immunoblotting both in the presence and absence of a pan caspase inhibitor qVD in order to distinguish changes that were specifically induced by 15d-PGJ2 from those that occurred secondarily to cell death. While the fission protein Drp1 migrated at ~80 kD in control cells, 15d-PGJ2 treatment resulted in the appearance of a slow migrating ~150 kD band within 2h, which persisted even after prolonged incubation (Fig. 4A). Fis1, which acts as a receptor for Drp1 on mitochondria did not show any significant change in its expression levels upon 15d-PGJ2 treatment (Fig. 4A). Decrease in Fis1 and Drp1 levels at 8h of 15d-PGJ2 treatment was blocked by the inclusion of a pan specific caspase inhibitor qVD (Fig. 4A).

Decrease in Mfn1 and Mfn2 protein levels, which started after 4h of 15d-PGJ2 treatment was not prevented by qVD (Fig. 4B). At least five different splice variant forms of the fusion protein OPA1 were identified in RPTC and they responded variably to 15d-PGJ2 treatment (Fig. 4B). While two long isoforms of OPA1 decreased progressively starting from 2h of 15d-PGJ2 treatment, the short isoforms showed an increase (Fig. 4B). Late appearance of additional slow migrating bands of OPA1 (Fig. 4B, indicated by an asterisk) suggested possible post translational modifications of OPA1 induced by 15d-PGJ2. Appearance of these slow migrating forms of OPA1 followed by the loss of larger isoforms of OPA1 (Fig. 4B) could be blocked by general transcription and translational inhibitors

(Supplemental Fig. 1) indicating that these forms of OPA1 are newly made. Interestingly, such slow migrating bands of OPA1 could also be seen by incubating cells with proteasomal inhibitor MG132 (supplemental Fig. 2) suggesting that these protein forms may be ubiquitinated. To confirm ubiquitination, OPA1 was immunoprecipitated from control and 15d-PGJ2-treated cell extracts and analyzed by immunoblotting with anti-OPA1 and anti-ubiquitin antibodies. As shown in Fig. 4C, the new slow migrating isoforms of OPA1 were recognized by anti-ubiquitin antibody suggesting that newly made OPA1 isoforms in 15d-PGJ2 treated cells are ubiquitinated. In consistence with our previous report [1], where the thiol antioxidants NAC and NMPG blocked mitochondrial elongation, also prevented disappearance of slower migrating isoforms as well as new synthesis and ubiquitination of OPA1 (Fig. 4D). These late changes of OPA1 and other fusion proteins could conceivably explain the inability of elongated mitochondria to maintain their tubular structure and their conversion to swollen structures with disorganized cristae.

DISCUSSION

We report here that 15d-PGJ2, an arachidonic acid metabolite, which inhibits Drp1 function and initially causes mitochondrial fusion [1], also induced late molecular and biochemical changes in mitochondrial fusion proteins leading to mitochondrial remodeling. Specifically, elongated mitochondria underwent a structural transformation to swollen disorganized structures (Fig 1B). In this study, we report several new findings. First, we have demonstrated two major mitochondrial shape transitions in response to 15d-PGJ2 treatment through regulation of fission and fusion proteins. Our results also indicate that mitochondrial elongation through inhibition of fission process by 15d-PGJ2 still required the presence of both outer and inner membrane fusion mediators, Mfn1, Mfn2 and OPA1 as mitochondria from OPA1-null or Mfn1/2 double null MEFs failed to undergo fusion. Interestingly, presence of either Mfn1 or Mfn2 alone allowed mitochondrial fusion by 15d-PGJ2 suggesting possible complementation between these two isoforms. The formation of large swollen mitochondria after initial elongation could probably be due to inactivation or loss of the fusion proteins. Consistent with this hypothesis, formation of large swollen mitochondria was preceded by a decrease in the levels of Mfn1, Mfn2 and loss of larger isoforms of OPA1 (Fig. 4B).

Notably, we show two major changes of OPA1 with 15d-PGJ2. First, there is proteolysis of OPA1 larger isoforms with concomitant increase in shorter isoforms. Secondly, there is synthesis of new OPA1 isoforms along with their ubiquitination. Accumulation of ubiquitinated proteins in the presence of 15d-PGJ2 was shown before [14], which is probably due to inhibition of proteasomes [15]. Ubiquitinated OPA1 is predominantly present in the NP40 detergent insoluble fraction suggesting possible aggregation of ubiquitinated OPA1. Inhibition of the fission process by inactivation of Drp1 by 15d-PGJ2 may stimulate proteolysis of fusion proteins, particularly that of OPA1. However, newly synthesized OPA1 is immediately being modified by ubiquitination during 15d-PGJ2 treatment. Proteolysis of larger isoforms and ubiquitination and aggregation of newly synthesized OPA1 appears to have affected the integrity of cristae resulting in the loss of tubular structure of mitochondria and formation of large swollen structures. Disruption of the oligomeric state of OPA1 as was observed by BN-PAGE (Fig. 3C) and disorganization of cristae structure, support the idea that OPA1 oligomers may maintain the cristae integrity. Destabilization of the inner mitochondrial membrane and disorganization of the mitochondrial cristae network are hypothesized to occur also with the *OPA1* mutations in dominant optic atrophy [16], which results in abnormal assembly and function of the mitochondrial respiratory complexes affecting ATP synthesis [17]. Mitochondrial remodeling through above biochemical events during 15d-PGJ2 treatment appears to have significantly impaired oxidative phosphorylation and ATP synthesis. Especially, when cells

were forced to use oxidative metabolism in glucose-free medium, cellular ATP levels were dramatically decreased (Fig. 3B). Thiol antioxidant NAC and NMPG which were reported previously to block mitochondrial elongation and stabilization of Drp1 oligomers, also prevented loss of OPA1 isoforms and their synthesis and ubiquitination (Fig. 4D), which further supports our hypothesis that inactivation of fission protein by any means may subsequently lead to the decreased activity of fusion proteins which would then affect mitochondrial function and dynamics. Our results clearly warn investigators of unintended consequences of using drugs that block mitochondrial fission to prevent apoptosis.

CONCLUSION

This finding shows for the first time novel changes in fusion protein OPA1 following inactivation or inhibition of a fission protein, which can provide clues to understand basis of diseases caused by an imbalance of fission or fusion events and thus can help to design treatment regimens for such conditions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Mishra N, Kar R, Singha PK, Venkatachalam MA, McEwen DG, Saikumar P. Inhibition of mitochondrial division through covalent modification of Drp1 protein by 15 deoxy-Delta(12,14)-prostaglandin J2. *Biochem Biophys Res Commun.* 2010; 395:17–24. [PubMed: 20307494]
2. Hoppins S, Lackner L, Nunnari J. The machines that divide and fuse mitochondria. *Annual review of biochemistry.* 2007; 76:751–780.
3. Chan DC. Mitochondrial fusion and fission in mammals. *Annual review of cell and developmental biology.* 2006; 22:79–99.
4. Smirnova E, Griparic L, Shurland DL, van der Bliek AM. Dynamin-related protein Drp1 is required for mitochondrial division in mammalian cells. *Mol Biol Cell.* 2001; 12:2245–2256. [PubMed: 11514614]
5. James DI, Parone PA, Mattenberger Y, Martinou JC. hFis1, a novel component of the mammalian mitochondrial fission machinery. *J Biol Chem.* 2003; 278:36373–36379. [PubMed: 12783892]
6. Santel A, Fuller MT. Control of mitochondrial morphology by a human mitofusin. *J Cell Sci.* 2001; 114:867–874. [PubMed: 11181170]
7. Cipolat S, Martins de Brito O, Dal Zilio B, Scorrano L. OPA1 requires mitofusin 1 to promote mitochondrial fusion. *Proc Natl Acad Sci U S A.* 2004; 101:15927–15932. [PubMed: 15509649]
8. Song Z, Chen H, Fiket M, Alexander C, Chan DC. OPA1 processing controls mitochondrial fusion and is regulated by mRNA splicing, membrane potential, and Yme1L. *J Cell Biol.* 2007; 178:749–755. [PubMed: 17709429]
9. Chen H, Chomyn A, Chan DC. Disruption of fusion results in mitochondrial heterogeneity and dysfunction. *J Biol Chem.* 2005; 280:26185–26192. [PubMed: 15899901]
10. Ishihara N, Nomura M, Jofuku A, Kato H, Suzuki SO, Masuda K, Otera H, Nakanishi Y, Nonaka I, Goto Y, Taguchi N, Morinaga H, Maeda M, Takayanagi R, Yokota S, Mihara K. Mitochondrial fission factor Drp1 is essential for embryonic development and synapse formation in mice. *Nature cell biology.* 2009; 11:958–966.

11. Parone PA, Da Cruz S, Tondera D, Mattenberger Y, James DI, Maechler P, Barja F, Martinou JC. Preventing mitochondrial fission impairs mitochondrial function and leads to loss of mitochondrial DNA. *PLoS ONE*. 2008; 3:e3257. [PubMed: 18806874]
12. Weinberg JM, Saikumar P. Mitochondrial function. *Methods in molecular medicine*. 2003; 86:351–371. [PubMed: 12886781]
13. Lee YJ, Jeong SY, Karbowski M, Smith CL, Youle RJ. Roles of the mammalian mitochondrial fission and fusion mediators Fis1, Drp1, and Opa1 in apoptosis. *Mol Biol Cell*. 2004; 15:5001–5011. [PubMed: 15356267]
14. Kar R, Singha PK, Venkatachalam MA, Saikumar P. A novel role for MAP1 LC3 in nonautophagic cytoplasmic vacuolation death of cancer cells. *Oncogene*. 2009; 28:2556–2568. [PubMed: 19448671]
15. Shibata T, Yamada T, Kondo M, Tanahashi N, Tanaka K, Nakamura H, Masutani H, Yodoi J, Uchida K. An endogenous electrophile that modulates the regulatory mechanism of protein turnover: inhibitory effects of 15-deoxy-Delta 12,14-prostaglandin J2 on proteasome. *Biochemistry*. 2003; 42:13960–13968. [PubMed: 14636064]
16. Lodi R, Tonon C, Valentino ML, Iotti S, Clementi V, Malucelli E, Barboni P, Longanesi L, Schimpf S, Wissinger B, Baruzzi A, Barbiroli B, Carelli V. Deficit of in vivo mitochondrial ATP production in OPA1-related dominant optic atrophy. *Annals of neurology*. 2004; 56:719–723. [PubMed: 15505825]
17. Zanna C, Ghelli A, Porcelli AM, Karbowski M, Youle RJ, Schimpf S, Wissinger B, Pinti M, Cossarizza A, Vidoni S, Valentino ML, Rugolo M, Carelli V. OPA1 mutations associated with dominant optic atrophy impair oxidative phosphorylation and mitochondrial fusion. *Brain*. 2008; 131:352–367. [PubMed: 18222991]

Figure 1. Effect of Drp1 deficiency on fusion protein expression and effect of prolonged Drp1 inhibition by 15d-PGJ2 on mitochondrial morphology

A) Expression of Drp1, OPA1, Mfn1 and Mfn2 proteins by western blotting in Wild type and Drp1 knockdown RPTC lysates probed with appropriate primary antibodies described in the experimental section. B) Changes in mitochondrial morphology from punctiform in untreated (0h) to elongated and interconnected structures at 2h, to large round structures at 4h and 6h treatment with 20 μ M 15d-PGJ2. At 8h most cells were fragmented and floating. Bar: 10 μ m. C) Number of cells with punctiform, elongated and interconnected tubular, and large spherical structures were scored after treating with 20 μ M 15d-PGJ2 at 0, 2, 4 and 6 h time points. Data represent the average $\pm$ SD of 3 independent experiments with all cells (at least 100) counted in several fields for each time point.

Figure 2. Requirement of Mitofusins and OPA1 in 15d-PGJ2 induced mitochondrial elongation

A) Total cell lysates from above MEFs were subjected to Western blotting using specific antibodies to evaluate fusion protein expression. Asterisk (*) represents a non-specific band reacts with Mfn2 antibody. B) Wild type, OPA1-null and Mfn1/2 double knockout MEF cells and C) single knock out cells (Mfn1-null and Mfn2-null) were loaded with mitotracker red dye and were treated with 15d-PGJ2 for indicated time periods for analyzing mitochondrial morphology using confocal microscopy as described in Experimental methods section. Bar: 10 μ m.

Figure 3. Loss of mitochondrial structure, function and molecular assembly in cells treated with 15d-PGJ2

A) Electron microscopic images showing changes in mitochondrial structure in RPTC treated with 15d-PGJ2 for indicated time periods. Magnification: 30,000 X. B) Total cellular ATP levels measured in RPTC that were grown in either regular medium containing glucose or oxidative substrate-rich and glucose-free medium at different time points of treatment with 15d-PGJ2. C) Analysis of OPA1 complexes by immunoblotting separated by BN-PAGE in control and 15d-PGJ2 treated RPTC.

Figure 4. 15d-PGJ2-mediated expression and modification of mitochondrial fusion and fission proteins

A) Western blotting analysis of total cell lysates for fission proteins Drp1 and Fis1 and B) fusion proteins Mfn1, Mfn2 and OPA1 upon treatment with 15d-PGJ2 in the absence or presence of qVD for indicated times. Asterisk (*) indicates appearance of new slow migrating forms of OPA1. C) Western blot showing ubiquitination of OPA1 in 15d-PGJ2 treated cells. Treated and untreated RPTC lysates obtained by SDS extraction were diluted with NP-40 buffer and subjected to immunoprecipitation with α -OPA1 antibody followed by immunoblotting with α -OPA1 and α -ubiquitin antibodies. D) RPTCs were pretreated with the thiol antioxidants NAC (10mM) or NMPG (5mM) for 1hr before addition of 15d-PGJ2. Whole cell lysates were probed for OPA1 by western blotting. Asterisk (*) indicates new ubiquitinated isoforms of OPA1.