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Inhibition of mitoNEET induces Pink1-Parkin-mediated mitophagy

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MitoNEET, a mitochondrial outer membrane protein containing the Asn-Glu-Glu-Thr (NEET) sequence, controls the formation of intermitochondrial junctions and confers autophagy resistance. Moreover, mitoNEET as a mitochondrial substrate undergoes ubiquitination by activated Parkin during the initiation of mitophagy. Therefore, mitoNEET is linked to the regulation of autophagy and mitophagy. Mitophagy is the selective removal of the damaged or unnecessary mitochondria, which is crucial to sustaining mitochondrial quality control. In numerous human diseases, the accumulation of damaged mitochondria by impaired mitophagy has been observed. However, the therapeutic strategy targeting of mitoNEET as a mitophagy-enhancing mediator requires further research. Herein, we confirmed that mitophagy is indeed activated by mitoNEET inhibition. CCCP (carbonyl cyanide m-chlorophenyl hydrazone), which leads to mitochondrial depolarization, induces mitochondrial dysfunction and superoxide production. This, in turn, contributes to the induction of mitophagy; mitoNEET protein levels were initially increased before an increase in LC3-Ⅱ protein following CCCP treatment. Pharmacological inhibition of mitoNEET using mitoNEET Ligand-1 (NL-1) promoted accumulation of Pink1 and Parkin, which are mitophagy-associated proteins, and activation of mitochondria–lysosome crosstalk, in comparison to CCCP alone. Inhibition of mitoNEET using NL-1, or mitoNEET shRNA transfected into RAW264.7 cells, abrogated CCCP-induced ROS and mitochondrial cell death; additionally, it activated the expression of PGC-1α and SOD2, regulators of oxidative metabolism. In particular, the increase in PGC-1α, which is a major regulator of mitochondrial biogenesis, promotes mitochondrial quality control. These results indicated that mitoNEET is a potential therapeutic target in numerous human diseases to enhance mitophagy and protect cells by maintaining a network of healthy mitochondria. [BMB Reports 2022; 55(7): 354-359]

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INTRODUCTION

Mitochondria are highly dynamic organelles that undergo structural remodeling in a well-regulated manner. Mitochondrial quality control (MQC) including dynamics and mitophagy is the important process for maintenance of mitochondrial function and structure (1, 2). Especially, mitophagy, the selective mitochondrial autophagy, is pivotal in controlling metabolic homeostasis and maintaining mitochondrial quality and function. These roles are critical in molecular events, such as oxidative stress, that lead to age-related dysfunctions development and subsequent disease progression (3, 4). Impaired mitophagy and cumulative mitochondrial dysfunction are closely linked to multiple human diseases, as supported by considerable evidence, such as age-related neurodegenerative diseases, metabolic diseases, muscle dystrophy, and liver disease (4, 5). The initial stage of mitophagy is stimulated by kinases such as 5′-AMP-activated protein kinase (AMPK), unc-51 like autophagy activating kinase 1 (Ulk1), and PTEN-induced putative kinase 1 (Pink1). The kinases promote each other and stimulate a downstream regulation of autophagy (6, 7). Consequently, Pink1–Parkin-mediated mitophagy, receptor-mediated mitophagy, or adaptor protein-mediated mitophagy is triggered (8). The polyubiquitination of various mitochondrial outer membrane proteins by Parkin induces the recognition of several adaptor molecules and microtubule-associated protein 1 light chain 3 (LC3) and the recruitment of autophagic machinery (6, 8, 9). The mitophagy-enhancing process facilitates the elimination of damaged mitochondria and is the basis for developing therapeutic strategies for several diseases. However, the molecular mechanism governing mitophagy remains unclear.

Current studies have demonstrated that mitoNEET, a protein exposed at the cytosolic surface of the mitochondria, controls the formation of intermitochondrial junctions and mitochondrial network morphology (10). Therefore, the deletion of mitoNEET alters the integrity of inter-mitochondrial junctions (11). Previous studies showed that mitoNEET is concerned in the regulation of autophagy signaling and it prevents autophagy (12, 13). In cells with reduced mitoNEET expression, proteins involved in autophagy accumulate; additionally, electron microscopy revealed autophagosome accumulation (12, 14). Furthermore, mitoNEET, along with other integral mitochondrial proteins in the outer mitochondrial membrane, undergoes ubiquitination during mito-

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phagy. Subsequently, the integral mitochondrial proteins containing mitoNEET are phosphorylated by Pink1 and reduced by E3 ubiquitin protein ligase (Parkin) recruitment during the early stages of mitophagy (7, 15, 16). These studies demonstrated that mitoNEET acts as a direct substrate of Parkin in the recruitment of autophagy adaptors and the elimination of mitoNEET is necessary for mitophagy initiation (16, 17). However, whether targeting mitoNEET enhances mitophagy has not been studied yet. Herein, we showed that mitoNEET inhibition promotes the accumulation of mitophagy-linked proteins and activation of the crosstalk between lysosomes and mitochondria during CCCP-induced mitochondrial damage. Thus, mitoNEET plays a conclusive role in mitophagy regulation, which has been clearly demonstrated in the results of the previous studies. Therefore, we hypothesized that mitoNEET is a potential molecular therapeutic target for several diseases through the elimination of damaged mitochondria and maintenance of mitochondrial quality control.

RESULTS

CCCP-mediated mitochondrial depolarization induced mitoNEET expression in macrophages

Mitochondrial depolarization due to environmental stress such as CCCP, a mitochondrial oxidative phosphorylation uncoupler, induces mitophagy-mediated clearance of damaged mitochondria or activates apoptosis. Therefore, CCCP is an indirect mitophagy and apoptosis inducer (4). In the present study, we hypothesized that mitoNEET contributes to faulty mitophagy in mitochondrial dysfunction diseases. To elucidate the role of mitoNEET in autophagy function during CCCP-induced selective autophagy of the mitochondria, we assayed mitoNEET expression during CCCP treatment. We harvested total protein at 0.5, 1, 3, 6, 9, and 12 hours after CCCP administration in RAW264.7 cells (Fig. 1A). MitoNEET protein expression was significantly increased at 1 and 3 hours prior to the conversion of LC3-I to LC3-Ⅱ protein. Additionally, 6 hours after CCCP treatment, LC3-Ⅱ protein levels increased, with decreased mitoNEET protein levels. These results indicated that mitoNEET is involved in the initial stages of autophagy. To study the function of mitoNEET in autophagy signaling, we analyzed the expression of autophagosomal marker LC3-Ⅱ and autophagy adaptor protein p62/SQSTM in mitoNEET inhibition by inhibitor NL-1. We found that NL-1 enhanced the expression levels of LC3-Ⅱ and p62 protein in cells exposed to CCCP (Fig. 1B), followed by a rapid decrease at 12 hours. To examine whether CCCP-induced expression of mitoNEET alters autophagy responses, we generated mitoNEET shRNA- or control shRNA-expressing cells. Expression levels of LC3-II and p62 protein in cells of shRNA-mediated knock-down of mitoNEET were higher than those in control cells (Fig. 1C), and then decreased rapidly at 12 hours. These results demonstrated that the elimination of mitoNEET is necessary for autophagy activation. Moreover, mitoNEET inhibition using inhibitor or shRNA could activate the CCCP-induced autophagy response. To verify that the inhibition of mitoNEET induces mitophagy,

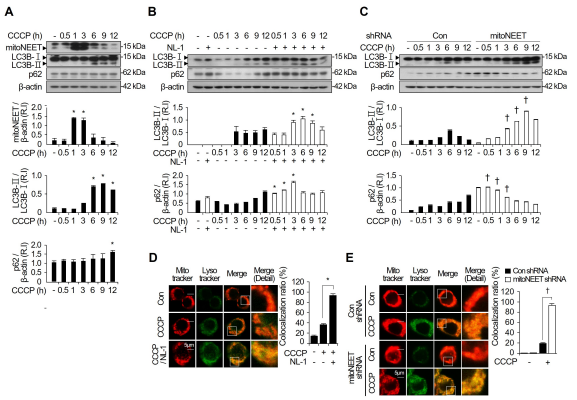

Fig. 1. Inhibition of mitoNEET increases CCCP-induced autophagy of the mitochondria. Total protein was extracted from RAW264.7 cells, Control shRNA-expressing or mitoNEET shRNA-expressing RAW264.7 cells after administration of vehicle or CCCP (25 μM) or CCCP plus NL-1 (20 μM) for variable time points. Expression levels of mitoNEET (A) and LC3-Ⅱ, p62 proteins (A-C) were assessed by western blotting. CCCP-induced colocalization of mitochondria and lysosome was assayed at 6 hours after by confocal fluorescence microscopy using the fluorescent probes LysoTracker (green fluorescence) and MitoTracker (red fluorescence) (D, E). Scale bar: 5 μm. Fluorescence intensity was measured using Image J. All data are expressed as the mean ± SD for the three independent experiments. *$P < 0.05$ for CCCP vs. CCCP plus NL-1. †$P < 0.05$ for control shRNA-expressing cells vs. mitoNEET shRNA-expressing cells in the presence of CCCP.

we assessed colocalization of lysosomes and mitochondria by confocal microscopy using MitoTracker Deep Red, and LysoTracker Green. (Fig. 1D, E). Confocal microscopy images showed that NL-1 or mitoNEET shRNA increased CCCP-induced colocalization of mitochondria and lysosomes when compared with CCCP alone. Taken together, these results indicated that inhibiting mitoNEET induces recruitment of autophagosomes and the selective degradation of mitochondria during CCCP-induced mitochondrial depolarization.

Inhibition of mitoNEET activated Pink1–Parkin-mediated mitophagy during CCCP-induced mitochondrial depolarization

Prior studies demonstrated that CCCP induces protein kinase (AMPK) activation and promotes mitochondrial fission, manifesting as heightened Pink1 and Parkin E3 ligase activity toward mitoNEET (7, 17). However, whether targeting of mitoNEET plays a role as an activator of Pink1–Parkin-mediated mitophagy remains unknown. To confirm that CCCP induces AMPK and mitophagy-associated molecules Pink1 and Parkin, we examined protein levels using western blot analysis in CCCP-treated RAW264.7 cells (Fig. 2A). Phosphate AMPKα protein expression levels were significantly increased in the early stages compared with the corresponding expression levels in the vehicle, followed by a rapid decrease. In addition, Pink1 protein levels began to increase 1 hour following CCCP administration, and there was a corresponding sequential increase in Parkin expression. We

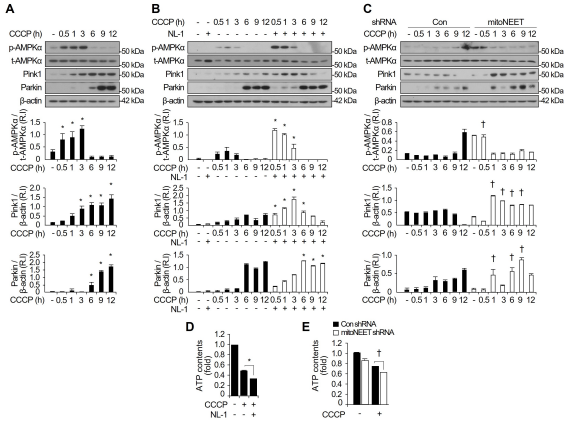

Fig. 2. Inhibition of mitoNEET increases CCCP-induced Pink1–Parkin-mediated mitophagy. RAW264.7 cells, control shRNA-expressing or mitoNEET shRNA-expressing cells were treated with vehicle, CCCP (25 μM), or CCCP plus NL-1 (20 μM) at variable time points. Total protein was harvested and the expression levels of mitophagy-related proteins, Total- or phosphate-AMPKα and Pink1, Parkin were analyzed by western blotting (A-C). Intracellular ATP levels were detected by ATP Assay Kit at 1 hour (D, E). All data are expressed as the mean ± SD for the three independent experiments. *P < 0.05 for CCCP vs. CCCP plus NL-1. †P < 0.05 for control shRNA-expressing cells vs. mitoNEET shRNA-expressing cells in the presence of CCCP.

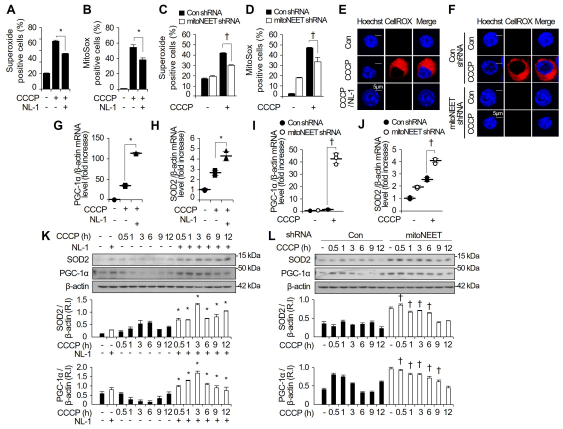

Fig. 3. Inhibition of mitoNEET attenuates CCCP-induced mitochondrial reactive oxygen species and enhances the expression of antioxidant defense genes. RAW264.7 cells, control shRNA-expressing or mitoNEET shRNA-expressing cells were treated with vehicle, CCCP (70 μM) or CCCP plus NL-1 (20 μM) (A-F). Superoxide (A, C) and mitoSOX (B, D) were measured at 9 hours by flow cytometry using the fluorescent probes superoxide detection reagent (orange) and mitoSOX mitochondrial superoxide indicator (red), respectively. Cytosolic ROS (E, F) was assayed at 6 hours for confocal fluorescence microscopy using the fluorescent probes and Hoechst 33258 (nuclei, blue) and CellROX® Deep Red (total ROS, red). Scale bar: 5 μm. Total RNA and protein were harvested 12 hours later, followed by measurement of mRNA encoding PGC-1α, SOD2 by quantitative RT-PCR (G-J), and expression levels of protein were detected by western blotting (K, L). All data are expressed as the mean ± SD for the three independent experiments. *P < 0.05 for CCCP vs. CCCP plus NL-1. †P < 0.05 for control shRNA-expressing cells vs. mitoNEET shRNA-expressing cells in the presence of CCCP.

showed that mitoNEET inhibition using inhibitor or shRNA augments autophagy marker expression and colocalization of lysosomes and mitochondria following CCCP treatment (Fig. 1). To verify whether mitoNEET inhibition activates CCCP-induced Pink1–Parkin-mediated mitophagy, we examined the expression levels of phosphate AMPKα, Pink1, and Parkin protein in CCCP-treated RAW264.7 cells in the presence or absence of NL-1 (Fig. 2B). We found that NL-1 enhanced the expression levels of phosphate AMPKα, Pink1, and Parkin proteins. Consistent with this, AMPKα, Pink1, and Parkin protein levels in cells of shRNA-mediated knock-down of mitoNEET were higher than the corresponding levels in control cells (Fig. 2C). These results demonstrated that mitoNEET inhibition using inhibitor or shRNA activated mitophagy through the upregulation of mitophagy-associated genes in the Pink1–Parkin pathway during CCCP-induced mitochondrial depolarization. CCCP is a strong inducer of AMPK through inhibition of ATP (adenosine triphosphate) synthesis (7, 18). Additionally, mitoNEET function is associated with ATP synthase, and pyruvate dehydrogenase, enzymes involved in the β-oxidation of fatty acids (19). We hypothesized that activated phosphate AMPKα in mitoNEET inhibition is generated by suppressing ATP synthesis during CCCP treatment, and assessed intracellular ATP levels using an ATP Assay Kit. We found that NL-1 reduced the intracellular ATP levels in cells treated to CCCP for 1 hour (Fig. 2D). Consistent with this, intracellular ATP levels in cells expressing mitoNEET shRNA were decreased in comparison with the corresponding levels in the control shRNA-expressing cells in the presence of CCCP (Fig. 2E). These results indicated that inhibiting mitoNEET in RAW264.7 cells promotes CCCP-mediated inhibition of ATP synthesis and enhances the phosphate AMPKα levels. This leads to stimulation of Pink1–Parkin during CCCP-induced mitochondrial damage. Taken together, these results indicate that targeting mitoNEET stimulates Pink1–Parkin, which acts as a downstream regulator of mitophagy, via phosphate AMPKα during CCCP-induced mitochondrial damage.

Inhibition of mitoNEET attenuated CCCP-induced mitochondrial ROS

CCCP induces superoxide production through mitochondrial depolarization (20). It has been postulated that mitochondrial dysfunction-induced reactive oxygen species (ROS) govern the pathogenesis of various diseases. Mitophagy activated in response to mitochondrial depolarization decreases ROS generation through the removal of damaged mitochondria (21). We evaluated whether mitophagy enhanced by inhibiting mitoNEET attenuates CCCP-induced mitochondrial ROS. Superoxide levels by CCCP (70 μM) were analyzed using flow cytometry and decreased by NL-1 administration at 9 hours (Fig. 3A). Additionally, mitoSOX levels were assessed by the mitoSOX mito-

chondrial superoxide indicator. The CCCP-induced mitoSOX level decreased following mitoNEET inhibitor, NL-1 (Fig. 3B). Furthermore, CCCP-induced superoxide and mitoSOX levels were reduced in mitoNEET shRNA expressing RAW264.7 cells (Fig. 3C, D). In addition, confocal microscopy clearly demonstrated that mitoNEET inhibition suppressed cytosolic ROS following treatment of CCCP (Fig. 3E, F).

To confirm the antioxidant effect of NL-1, during CCCP-induced ROS production, we examined the expression of PGC-1α (peroxisome proliferator-activated receptor gamma coactivator 1-alpha), as well as SOD2 (superoxide dismutase 2), in the presence or absence of NL-1. We found that mitoNEET inhibitor, NL-1, enhanced the mRNA (Fig. 3G, H) and protein (Fig. 3K) expression levels of PGC-1α and SOD2 in RAW264.7 cells. Consistent with this, PGC-1α, SOD2 mRNA (Fig. 3I, J), and protein (Fig. 3L) levels in cell of shRNA-mediated knock-down of mitoNEET were higher than those in the control cells. These results demonstrated that NL-1 or mitoNEET shRNA may attenuate CCCP-induced mitochondrial ROS production through the upregulation of the expression of antioxidant defense genes with enhanced mitophagy. Furthermore, PGC-1α, as a major regulator of mitochondrial biogenesis, maintains a network of healthy mitochondria by balancing the generation of new organelles and the removal of damaged mitochondria (8, 22). We did another experiment to demonstrate that mitoNEET inhibition induces the activation of mitochondrial biogenesis via the increase of PGC-1α and the activation of mitophagy via increase of Drp-1 (a fission protein) and decrease of mitofusin 2 (Mfn2; a fusion protein) in the presence with CCCP by western blot analysis. (Supplementary Fig. 1). These data indicated that by targeting mitoNEET, it is possible to sustain mitochondrial quality by maintaining a network of healthy mitochondria during the enhancement of mitophagy following CCCP treatment.

Inhibition of mitoNEET hampered CCCP-induced mitochondrial apoptosis

Mitochondria play key roles in activating apoptotic cell death in mammalian cells (23). Mitochondrial depolarization by CCCP induces ROS-mediated cell death (20). We showed that CCCP-induced mitochondrial ROS was attenuated by mitoNEET inhibition (Fig. 3). We assessed the effects of mitoNEET inhibition during CCCP-induced cell death using the Annexin V (AV) / Propidium Iodide (PI) Apoptosis Detection Kit. CCCP-induced apoptosis was rescued by mitoNEET inhibitor, NL-1, or mitoNEET shRNA at 12 hours (Fig. 4A, B) Moreover, mitoNEET inhibition decreased the levels of lactate dehydrogenase (LDH), the release of which serves as a cell-death marker (Fig. 4C, D). We treated cells with CCCP and then performed a cell-viability assay. Cell viability was evaluated by the MTT assay using the Ez-Cytox Cell viability Assay Kit. CCCP-induced cell death results indicated that inhibiting mitoNEET bestows a protective effect against CCCP-induced apoptosis through the attenuation of mitochondrial ROS via mitophagy. CCCP directly interferes with mitochondrial function and induces apoptosis (20). We

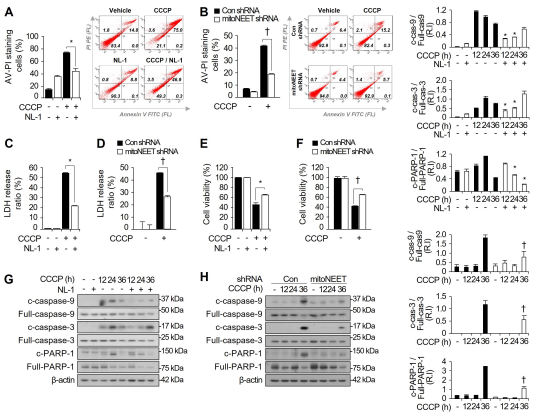

Fig. 4. Inhibition of mitoNEET hinders CCCP-induced mitochondrial apoptosis. RAW264.7 cells, control shRNA-expressing or mitoNEET shRNA-expressing cells were treated with vehicle, CCCP (25 μM) or CCCP plus NL-1 (20 μM). Cells were then stained with Annexin V-FITC/PI-PE and detected by flow cytometry at 12 hours (A, B). The LDH release was analyzed by the LDH-release assay at 12 hours (C, D). Cell viability was analyzed using Ez-Cytox Cell viability Assay Kit at 6 hours (E, F). All data are expressed as the mean ± SD from three independent experiments. *$P < 0.05$ for CCCP vs. CCCP plus NL-1. †$P < 0.05$ for control shRNA-expressing cells vs. mitoNEET shRNA-expressing cells in the presence of CCCP. Total protein was harvested 12, 24 and 36 hours after administration of vehicle, CCCP (10 μM) or CCCP plus NL-1 (20 μM) in RAW264.7 cells and control shRNA-expressing or mitoNEET shRNA-expressing RAW264.7 cells. Expression levels of mitochondrial apoptosis related proteins, cleaved-caspase-9, -caspase-3, and -PARP-1, were detected by western blotting (G, H).

hypothesized that cell death regulated by mitoNEET inhibition during CCCP-induced mitochondrial damage is mitochondrial apoptosis. We isolated protein from cells at 12, 24, and 36 hours after CCCP (10 μM) or NL-1 administration. Next, levels of mitochondrial apoptosis marker protein were determined using western blotting. CCCP-induced cleavage of caspase-9 (cysteine-aspartic proteases-9), caspase-3 (cysteine-aspartic proteases-3), and PARP-1 (Poly [ADP-ribose] polymerase 1) proteins were decreased in the response of NL-1 in RAW264.7 cells (Fig. 4G) and in mitoNEET shRNA expressing RAW264.7 cells (Fig. 4H). Taken together, these results demonstrated that mitoNEET serves as a key molecule in mediating mitochondrial cell death through the regulation of mitochondrial quality including the activation of mitophagy and PGC-1α, the antioxidant defense gene, following CCCP treatment.

DISCUSSION

Mitophagy, selective autophagy of mitochondria, is an important mitochondrial quality control mechanism that removes damaged mitochondria (1). In the current research study, defective mitophagy is emerging as a potential therapeutic target for the treatment of human diseases (4, 24). Early studies suggested that

mitoNEET, a mitochondrial outer membrane protein, plays a role in the intermitochondrial junction and in the regulation of autophagy or mitophagy (11, 12, 16). In the present study, we proposed that targeting mitoNEET is a potential enhancer of mitophagy in mitochondrial dysfunctional diseases. Our results showed that mitoNEET protein levels are induced prior to the elevation of LC3-Ⅱ and p62 levels, which are autophagy-related proteins, during CCCP treatment in RAW264.7 cells (Fig. 1). These results demonstrated that mitoNEET is operative in the early stage of the autophagic response. Prior studies reported that mitoNEET is significantly reduced in response to E3 ubiquitin protein ligase (Parkin) recruitment in CCCP-induced mitophagy (17). However, whether the targeting of mitoNEET plays a role as a mitophagy activator has not been investigated yet. Interestingly, our data showed that mitoNEET inhibition using inhibitor or shRNA augments autophagy-related proteins, in addition to the colocalization of mitochondria and lysosomes (Fig. 1). Mitophagy mediated by AMPK activation in response to stresses that deplete intracellular ATP in early time recovers ATP production and mitochondrial function (7, 25). In our results, with the early reduction of ATP, mitophagy-associated protein levels of phosphate AMPKα, Pink1, and Parkin were enhanced by mitoNEET inhibition (Fig. 2). Therefore, our results showed that inhibiting mitoNEET, which acts as a regulator of the intermitochondrial junction, could enhance Pink1–Parkin-mediated mitophagy. Recent studies have reported that mitoNEET is associated with mitochondrial dysfunction in disease progression. Mitochondrial dysfunction could induce mitophagy as a feedback mechanism to remove damaged mitochondria. A prior study advanced an opinion that mitoNEET is a mitophagy-activating molecule as suggested by accumulating autophagosomes caused by mitoNEET-mediated mitochondrial dysfunction (26). However, in several human diseases, inefficient mitophagy results in the accumulation of dysfunctional mitochondria and mitophagosome due to the decreased fusion with lysosomes and consequent failed mitochondrial quality control (4). In our study, we showed that LC3-II protein levels raised with the attenuation of mitoNEET protein levels, which were initially increased. In addition, mitoNEET inhibition promoted the accumulation of mitophagy-linked proteins and the activation of mitochondria–lysosome crosstalk. These findings demonstrated that mitoNEET-mediated dysfunctional mitochondria generation under stress conditions needs to be suppressed for the enhancement and initiation of mitophagy (27). Furthermore, mitoNEET inhibition using inhibitor or shRNA attenuated CCCP-induced ROS or superoxide (Fig. 3) and increased PGC-1α and SOD2 mRNA and protein levels (Fig. 4). Particularly, as PGC-1α is a major regulator of mitochondrial biogenesis, the balance of elevated PGC-1α and mitophagy assures maintenance of the mitochondrial contents (8, 22). CCCP-induced ROS, disequilibrium of mitochondrial homeostasis cause deterioration of mitochondrial function and cell death (22, 23). Our results showed that mitoNEET inhibition recovers CCCP-induced cell death (Fig. 4). These results indicated that targeting mitoNEET exerts a protective effect against cell stress through mitochondrial quality control with elevated mitophagy and the replacement by mitochondrial biogenesis. Therefore, our results delineated a new paradigm for the role of mitoNEET in the mitophagic process and demonstrated that mitoNEET is a potential therapeutic target for the elimination of irreversibly damaged mitochondria and maintaining mitochondrial quality control in a wide range of diseases.

MATERIALS AND METHODS

Details on the used methods are provided in the expanded Materials and Methods section in the online data supplement.

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CONFLICTS OF INTEREST

The authors have no conflicting interests.

CONTRIBUTIONS

Conceptualization, S.W.C., S-J.L.; Methodology, Seunghee Lee; Validation, Seunghee Lee, Sangguk Lee and S-J.L., S.W.C.; Formal Analysis, Seunghee Lee, Sangguk Lee; Investigation, Seunghee Lee, Sangguk Lee; Writing Original Draft Preparation, S.W.C., Seunghee Lee; Writing Review & Editing, S.W.C., Seunghee Lee; Visualization, Seunghee Lee; Supervision, S.W.C.; Project Administration, S.W.C.; Funding Acquisition, S.W.C., S-J.L.

REFERENCES

1. Ashrafi G, Schwarz TL (2013) The pathways of mitophagy for quality control and clearance of mitochondria. Cell Death Differ 20, 31-42
2. Tilokani L, Nagashima S, Paupe V, Prudent J (2018) Mitochondrial dynamics: overview of molecular mechanisms. Essays Biochem 62, 341-360
3. Um JH, Yun J (2017) Emerging role of mitophagy in human diseases and physiology. BMB Rep 50, 299-307
4. Doblado L, Lueck C, Rey C et al (2021) Mitophagy in human diseases. Int J Mol Sci 22, 3903
5. Lou G, Palikaras K, Lautrup S, Scheibye-Knudsen M, Tavernarakis N, Fang EF (2020) Mitophagy and neuroprotection. Trends Mol Med 26, 8-20
6. Gkikas I, Palikaras K, Tavernarakis N (2018) The role of mitophagy in innate immunity. Front Immunol 9, 1283
7. Hung CM, Lombardo PS, Malik N et al (2021) AMPK/ULK1-

mediated phosphorylation of Parkin ACT domain mediates an early step in mitophagy. Sci Adv 7, eabg4544
8. Park GH, Park JH, Chung KC (2021) Precise control of mitophagy through ubiquitin proteasome system and deubiquitin proteases and their dysfunction in Parkinson's disease. BMB Rep 54, 592-600
9. Shin WH, Park JH, Chung KC (2020) The central regulator p62 between ubiquitin proteasome system and autophagy and its role in the mitophagy and Parkinson's disease. BMB Rep 53, 56-63
10. Vernay A, Marchetti A, Sabra A et al (2017) MitoNEET-dependent formation of intermitochondrial junctions. Proc Natl Acad Sci U S A 114, 8277-8282
11. Molino D, Pila-Castellanos I, Marjault HB et al (2020) Chemical targeting of NEET proteins reveals their function in mitochondrial morphodynamics. EMBO Rep 21, e49019
12. Sohn YS, Tamir S, Song L et al (2013) NAF-1 and mitoNEET are central to human breast cancer proliferation by maintaining mitochondrial homeostasis and promoting tumor growth. Proc Natl Acad Sci U S A 110, 14676-14681
13. Salem AF, Whitaker-Menezes D, Howell A, Sotgia F, Lisanti MP (2012) Mitochondrial biogenesis in epithelial cancer cells promotes breast cancer tumor growth and confers autophagy resistance. Cell Cycle 11, 4174-4180
14. Tamir S, Paddock ML, Darash-Yahana-Baram M et al (2015) Structure–function analysis of NEET proteins uncovers their role as key regulators of iron and ROS homeostasis in health and disease. Biochim Biophys Acta 1853, 1294-1315
15. Yamano K, Matsuda N, Tanaka K (2016) The ubiquitin signal and autophagy: an orchestrated dance leading to mitochondrial degradation. EMBO Rep 17, 300-316
16. Lazarou M, Narendra DP, Jin SM, Tekle E, Banerjee S, Youle RJ (2013) PINK1 drives Parkin self-association and HECT-like E3 activity upstream of mitochondrial binding. J Cell Biol 200, 163-172
17. Seabright AP, Fine NHF, Barlow JP et al (2020) AMPK activation induces mitophagy and promotes mitochondrial fission while activating TBK1 in a PINK1-Parkin independent manner. FASEB J 34, 6284-6301
18. Herzig S, Shaw RJ (2018) AMPK: guardian of metabolism and mitochondrial homeostasis. Nat Rev Mol Cell Biol 19, 121-135
19. Colca JR, McDonald WG, Waldon DJ et al (2004) Identification of a novel mitochondrial protein ("mitoNEET") cross-linked specifically by a thiazolidinedione photoprobe. Am J Physiol Endocrinol Metab 286, E252-E260
20. Park JS, Kang DH, Bae SH (2015) p62 prevents carbonyl cyanide m-chlorophenyl hydrazine (CCCP)-induced apoptotic cell death by activating Nrf2. Biochem Biophys Res Commun 464, 1139-1144
21. Livingston MJ, Wang J, Zhou J et al (2019) Clearance of damaged mitochondria via mitophagy is important to the protective effect of ischemic preconditioning in kidneys. Autophagy 15, 2142-2162
22. Palikaras K, Lionaki E, Tavernarakis N (2015) Balancing mitochondrial biogenesis and mitophagy to maintain energy metabolism homeostasis. Cell Death Differ 22, 1399-1401
23. Lee S, Jeong Y, Roe J, Huh H, Paik SH, Song J (2021) Mitochondrial dysfunction induced by callyspongiolide promotes autophagy-dependent cell death. BMB Rep 54, 227-232
24. Bakula D, Scheibye-Knudsen M (2020) MitophAging: mitophagy in aging and disease. Front Cell Dev Biol 8, 239
25. Wang Bei, Nie J, Wu L et al (2019) AMPKα2 protects against the development of heart failure by enhancing mitophagy via PINK1 phosphorylation. Circ Res 122, 712-729
26. Kusminski CM, Chen S, Ye R et al (2016) MitoNEET-Parkin effects in pancreatic α- and β-cells, cellular survival, and intrainsular cross talk. Diabetes 65, 1534-1555
27. Lee S, Seok BG, Lee SJ, Chung SW (2022) Inhibition of mitoNEET attenuates LPS-induced inflammation and oxidative stress. Cell Death Dis 13, 127