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Mitochondrial chaperone DnaJA3 induces Drp1-dependent mitochondrial fragmentation

Adam N. Elwi[a,1], Byoungchun Lee[a,1], H. Christopher Meijndert[a], Janice E.A. Braun[b], Sung-Woo Kim[a,*]

[a] Southern Alberta Cancer Research Institute, Department of Biochemistry and Molecular Biology, University of Calgary, Alberta, Canada
[b] Hotchkiss Brain Institute, Department of Physiology and Pharmacology, University of Calgary, Alberta, Canada

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ABSTRACT

Mitochondrial morphology is dynamic and controlled by coordinated fusion and fission pathways. The role of mitochondrial chaperones in mitochondrial morphological changes and pathology is currently unclear. Here we report that altered levels of DnaJA3 (Tid1/mtHsp40) a mitochondrial member of the DnaJ protein family, and heat shock protein (Hsp) co-chaperone of matrix 70 kDa Hsp70 (mtHsp70/mortalin/HSPA9), induces mitochondrial fragmentation. Suppression of DnaJA3 induced mitochondrial fragmentation in HeLa cells. Elevated levels of DnaJA3 in normal Hs68 fibroblast cells and HeLa, SKN-SH, U87 and U251 cancer cell lines induces mitochondrial fragmentation. Mitochondrial fragmentation induction was not observed in HeLa cells when other DnaJA family members, or mitochondrial DnaJ protein HSC20, were ectopically expressed, indicating that the effects on mitochondrial morphology were specific to DnaJA3. We show that the DnaJ domain (amino acids 88–168) of DnaJA3 is sufficient for the induction of mitochondrial fragmentation. Furthermore, an H121Q point mutation of the DnaJ domain, which abrogates interaction and activation of mtHsp70 ATPase, eliminates fragmentation induced by DnaJA3. This suggests that DnaJA3 interaction with mtHsp70 may be critical in mitochondrial morphological changes. DnaJA3-induced mitochondrial fragmentation was dependent on fission factor dynamin-related protein 1 (Drp1). Ectopic expression of the mitofusins (Mfn1 and Mfn2), however, does not rescue DnaJA3-induced mitochondrial fragmentation. Lastly, elevated levels of DnaJA3 inducing mitochondrial fragmentation were associated with reduction in cell viability. Taken together, elevated DnaJA3 induces Drp1-depedendent mitochondrial fragmentation and decreased cell viability.

1. Introduction

During their life cycle, mitochondria fuse with each other and split apart continuously to maintain a filamentous network (Westermann, 2010). Dynamism and plasticity of mitochondrial morphology is crucial for efficient energy production and dissipation (Collins et al., 2002; Campello et al., 2006), maintenance of mitochondrial DNA (mtDNA; Chen et al., 2010), and unleashing the apoptotic cascade (Frank et al., 2001; Scorrano et al., 2002, Frezza et al., 2006). The morphogenesis machinery of mitochondria includes dynamin-related GTPases at outer membranes, dynamin-related protein 1 (Drp1; Smirnova et al., 2001; Cereghetti et al., 2008) and mitofusin 1 (Mfn1) and 2 (Mfn2; Koshiba et al., 2004) and inner membranes, optic atrophy 1 (Opa1; Cipolat et al., 2004). A striking change of mitochondrial morphology dubbed mitochondrial fragmentation occurs when fission factors are activated or fusion factors are inactivated (Frank et al., 2001; Scorrano et al., 2002; Frezza et al., 2006).

A host of chaperones are implicated in maintenance of protein quality control and protein function (Schmidt et al., 2010; Kampinga and Craig, 2010). DnaJ proteins recognize nascently-synthesized client polypeptides and deliver them to ATP-bound

Abbreviations: Aco1, aconitase; antA, antimycin A; CCCP, carbonyl cyanide *m*-chlorophenyl hyzdrazin; COXIV, cyclooxygenase IV; DsRed2, *Discosoma sp.* Red Fluorescent Protein; Drp1, dynamin-related protein 1; EGFP, Enhanced Green Fluorescent Protein; HBSS, Hank's Buffered Salt Solution; Hsp, heat shock protein; hHSC20/HscB/DnaJC20, human heat shock cognate protein 20-kDa; Glt1, glutamate synthase; Jac1p, J-type co-chaperone 1 protein; mtHsp40/Tid1/DnaJA3, matrix 40-kDa Hsp; mtHsp70/mortalin/HSPA9, matrix 70-kDa Hsp; MOCS1, molybdenum cofactor biosynthesis protein 1; mtDNA, mitochondrial DNA; Mfn1, mitofusin 1; mitoRFP, mitochondrial-targeted variant of DsRed2; Mfn2, mitofusin 2; MOI, multiplicity of infection; Opa1, optic atrophy 1; PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction; pRS, pRetroSuper; RIPA, radioimmunoprecipitation buffer; ROS, reactive oxygen species; siRNA, short interfering RNA; SDS, sodium dodecyl sulfate; SD, standard deviation; TMRM, tetramethyl rhodamine methyl ester; TIM14, translocase of inner mitochondrial membrane 14-kDa; TIM23, translocase of inner mitochondrial membrane 23-kDa; Tim44, translocase of inner mitochondrial membrane 4-kDa; TOM20, translocase of outer mitochondrial membrane 20-kDa.

* Corresponding author at: Department of Biochemistry and Molecular Biology, Southern Alberta Cancer Research Institute, University of Calgary, 3330 Hospital Drive NW, Calgary, Alberta, Canada T2N 4N1. Tel.: +1 403 220 3702; fax: +1 403 210 9663.
E-mail address: swkim@ucalgary.ca (S.-W. Kim).
[1] These authors contributed equally to this work.

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Hsp70 *via* DnaJ domain interaction, followed by ATP hydrolysis and client protein release by Hsp70 (Kampinga and Craig, 2010). Cycles of ATP-dependent binding and release afford client proteins the opportunity to fold correctly (Kampinga and Craig, 2010). Even though DnaJ domain-containing matrix 40 kDa heat shock protein (mtHsp40/Tid1/DnaJA3; Rowley et al., 1994) co-chaperone and matrix 70 kDa heat shock protein (mtHsp70/mortalin/HSPA9) chaperone were discovered over two decades ago, it still remains unclear how maintenance of the integrity and homogeneity of the mitochondrial proteome by mitochondrial chaperones contributes to mitochondrial homeostasis.

Mammals express two alternatively spliced forms of DnaJA3 (43 kDa and 40 kDa), which have an amino-terminal mitochondrial-targeting sequence, J domain, glycine/phenylalanine linker region, and a central cysteine-rich region resembling a zinc finger repeat. J domains are tetrahelical regions with a highly conserved tripeptide of histidine, proline and aspartic acid located between helices II and III that interact with and activate the Hsp70/Hsc70s (Kampinga and Craig, 2010). DnaJA3-null mice are embryonic lethal (Lo et al., 2004) and conditional deletion in heart leads to dilated cardiomyopathy, presumably through deleterious effects on function of mtDNA polymerase, Polga (Hayashi et al., 2006). Many lines of strong evidence suggest that mitochondrial dysfunction occurs early and is causally linked to various ageing-related diseases including neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease and Huntington's disease (Lin & Beal, 2006). Dysfunction of mitochondrial protein quality systems, including mitochondrial chaperone proteins, is emerging as key underlying feature of neurodegenerative conditions (Tatsuta & Langer, 2008). In addition, mtHsp70 mutations in Parkinson's disease are associated with mitochondrial morphology changes and mitochondrial dysfunction (Burbulla et al., 2010).

The role of DnaJA3 in mitochondrial morphology has not been studied and a more comprehensive analysis of mitochondrial-specific functions of DnaJA3 in mammalian cells is warranted. To test the whether DnaJA3 contributes to mitochondrial morphology, we assessed fragmentation of mitochondria following suppression or ectopic over-expression of DnaJA3 or DnaJA3 truncations and mutations. Our findings support a role for DnaJA3 in inducing mitochondrial fragmentation through the Drp1 fission pathway.

2. Experimental procedures

2.1. Ethics statement

Care of experimental animals described was in accordance with institutional guidelines.

2.2. Materials

All chemicals and reagents were from Sigma–Aldrich (ON, Canada) unless noted otherwise.

2.3. Molecular biology

Mitochondrial-targeted variant of *Discosoma sp.* Red Fluorescent Protein (DsRed2; mitoRFP) were cloned by inserting mitochondrial targeting sequence of cyclooxygenase IV (COXIV) in frame with pDsRed2-N1 (Clontech, CA, USA) by the annealing and ligating the following *NheI* and *BamHI* site-flanked oligonucleotides into pDsRed2-N1: 5′-(*NheI*)CTAGCATGTCCGTCCTGACGCCGCTGCTGCTGCGGGGCTTGACAGGCTCGGCCCGGCGGCTCCCAGTGCCGCGCGCCAAGATCCATTCGTTGGGG-3′ and 5′-(*BamHI*)ATCCCCCAACGAATGGATCTTGGCGCGCGGCACTGGGAGCCGCCGGGCCGAGCCTGTCAAGCCCCGCAGCAGCAGCGGCGTCAGGACGGACATG-3′. Retroviral expression construct for mitoRFP was generated by sub-cloning into pRetroSuper (pRS) vector (Origene, MD, USA). Adenovirus containing Enhanced Green Fluorescent Protein (EGFP) reporter and expression cassette for DnaJA3 long ($DnaJA3_L$), short ($DnaJA3_S$), and $DnaJA3_S$ mutants, including N-terminal domain deletion of mitochondrial targeting sequence and processing site ($DnaJA3_S\Delta$MTS; amino acids 89–453), C-terminal client protein binding domain deletion (DnaJA3ΔCT; amino acids 1–292), and DnaJ domain inactivating point mutation H121Q ($DnaJA3_S$HQ), or empty expression cassette as a control were described previously (Ahn et al., 2010). Human mitofusin 1- (Mfn1) and 2- (Mfn2) myc-tagged expression constructs were from David C. Chan (California Institute of Technology, CA, USA). Myc-tagged human heat shock cognate protein 20-kDa (hHSC20/HscB/DnaJC20) construct was from Tracey A. Rouault (National Institute of Health, Bethesda, MD, USA; Uhrigshardt et al., 2010). DnaJA1-, DnaJA2-, and DnaJA4-GFP fusion protein expression constructs were from H. Kampinga (University of Groningen, AD, The Netherlands; Hageman and Kampinga, 2009). DnaJA3- EGFP fusion protein expression construct was described previously (Trinh et al., 2010). Expression constructs were generated for full-length $DnaJA3_S$ and $DnaJA3_L$ in pRK5 containing expression cassette for EGFP reporter (pRK5-EGFP) and for DnaJA3 domain deletion mutants in pRK5 and pRK5-EGFP, including N-terminal domain deletion of mitochondrial targeting sequence and processing site ($DnaJA3_L\Delta$MTS; amino acids 89–480) and serial C-terminal domain deletions including C-terminal client protein binding (DnaJA3ΔCT; amino acids 1–292), the cysteine-rich (characterized by CXXC[XG]1–2 repeats) domain (CXXC) resembling a zing finger ($DnaJA3_{1-235}$; amino acids 1–235), and glycine- and phenylalanine- (G/F)-rich domain ($DnaJA3_{1-168}$; amino acids 1–168), by ligating polymerase chain reaction (PCR) products into pRK5 or pRK5-GFP, obtained using the following *XbaI* or *HindIII* site-flanked primers: 5′-(*XbaI*)ATGGCTGCGCGGTGCTCC-3′ and 5′-(*HindIII*)TCATGAGGTAAACATTTTCTT-3′ (for $DnaJA3_L$); 5′-(*XbaI*)TTGGCCAAAGAAGATTAT-3′ and 5′-(*HindIII*)TCATGAGGTAAACATTTTCTT-3′ (for $DnaJA3_L\Delta$MTS); 5′-(*XbaI*)ATGGCTGCGCGGTGCTCC-3′ and 5′-(*HindIII*)TCATGAGGTAAACATTTTCTT-3′ (for $DnaJA3_S$); 5′-(*XbaI*)ATGGCTGCGCGGTGCTCC-3′ and 5′-(*HindIII*)GCAGACCACACAGGGCG-3′ (for DnaJA3ΔCT); 5′-(*XbaI*)ATGGCTGCGCGGTGCTCC-3′ and 5′-(*HindIII*)CGTGTCCATGATGTTCAC-3′ (for $DnaJA3_{1-235}$); and 5′-(*XbaI*) ATGGCTGCGCGGTGCTCC-3′ and 5′-(*HindIII*)GCCGCTGGCCCCAGGATC-3′ (for $DnaJA3_{1-168}$). Expression construct for $DnaJA3_L$ containing inactivating DnaJ domain point mutation H121Q ($DnaJA3_L$HQ) was from K. Münger (Harvard Medical School, MA, USA; Schilling et al., 1998). Short interfering RNAs (siRNAs) against the DnaJA3 sequence, 5′-CCGGATTAACAGCTACGGCTA-3′, the Drp1 sequence, 5′-CAGGAGCCAGCTAGATATTAA-3′, and non-silencing siRNA were from QIAGEN (ON, Canada).

2.4. Cell culture

Normal human skin fibroblast primary cultures (Hs68) cells and neuroblastoma cell line SKN-SH were from D. Senger (University of Calgary, AB, Canada), glioblastoma cell lines (U87, U251) were from P. Forsyth (University of Calgary), and human cervical carcinoma cell line (HeLa) was from E. Kurz (University of Calgary). Cells were cultured in high-glucose Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum in a humidified atmosphere containing 5% CO_2 at 37 °C, with weekly sub-culturing using trypsin-EDTA detachment (0.05% trypsin, 2 mM EDTA). For experiments, cells (10^5) were cultured on 6-well tissue culture plates, or 6-well plates with #1.5 22 mm × 22 mm glass coverslips, and treated as indicated. Adenoviral infections were carried out at indicated multiplicity of infections (MOIs). DNA transfections were carried out using Lipofectamine 2000 (Invitrogen, ON, Canada) with 0.5 μg DNA amounts and siRNA transfections were