Dynamin-Related Protein 1 Translocates from the Cytosol to Mitochondria during UV-Induced Apoptosis

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ABSTRACT

Mitochondria are dynamic structures that frequently divide and fuse with one another to maintain their architecture and functions [1]. Mitochondrial fission and fusion are normal events in healthy cells. While the precise mechanism for the shape changes is not well understood, perturbations of these processes affect cell survival and the evolutionarily conserved proteins mediate these processes [5], indicating that mitochondrial fission and fusion are important for cellular functions. The dynamin-related GTPase Drp1 is an evolutionarily conserved protein that contributes to outer mitochondrial membrane fission in mammalian cells and its functional impairment results in elongated mitochondria [3]. Similar to other dynamins, Drp1 contains an N-terminal GTPase domain, a middle domain involved in protein self-assembly and a C-terminal GTPase effector domain [4-7]. In vitro, DRP1 assembles into spirals at division sites around the outer mitochondrial membrane and tubulates liposomes in a nucleotide-dependent manner, suggesting that it functions directly in membrane constriction and/or scission [8]. In vivo, DRP1 forms cytosolic tetramers and is recruited to defined loci along the mitochondrial surface, which often mark sites of mitochondrial fission [3,5,6,9]. Drp1 exists primarily in the cytoplasm but partially associates into defined foci on the outer surface of mitochondria that coalesce at sites of organelle fission [3].

UV irradiation is a DNA-damaging agent that activates a p53-dependent apoptotic response to induce apoptosis [10]. Our previous studies demonstrate that Bax translocation induced by UV irradiation is a p53 transcription-dependent event and PUMA promotes Bax translocation both by directly interacting with Bax and by competitive binding to Bcl-XL in UV-induced apoptosis [11-12]. Furthermore, BimL is involved in Bax activation during UV irradiation-induced apoptosis [13-14]. These results indicated that UV-induced apoptosis is a highly complex process.

Keywords: Drp1, fission, UV, apoptosis

1 INTRODUCTION

Mitochondria are dynamic organelles that frequently divide and fuse with one another to maintain their architecture and functions [1]. Mitochondrial fission and fusion are normal events in healthy cells. While the precise mechanism for the shape changes is not well understood, perturbations of these processes affect cell survival and the evolutionarily conserved proteins mediate these processes [5], indicating that mitochondrial fission and fusion are important for cellular functions. The dynamin-related GTPase Drp1 is an evolutionarily conserved protein that contributes to outer mitochondrial membrane fission in mammalian cells and its functional impairment results in elongated mitochondria [3]. Similar to other dynamins, Drp1 contains an N-terminal GTPase domain, a middle domain involved in protein self-assembly and a C-terminal GTPase effector domain [4-7]. In vitro, DRP1 assembles into spirals at division sites around the outer mitochondrial membrane and tubulates liposomes in a nucleotide-dependent manner, suggesting that it functions directly in membrane constriction and/or scission [8]. In vivo, DRP1 forms cytosolic tetramers and is recruited to defined loci along the mitochondrial surface, which often mark sites of mitochondrial fission [3,5,6,9]. Drp1 exists primarily in the cytoplasm but partially associates into defined foci on the outer surface of mitochondria that coalesce at sites of organelle fission [3].

UV irradiation is a DNA-damaging agent that activates a p53-dependent apoptotic response to induce apoptosis [10]. Our previous studies demonstrate that Bax translocation induced by UV irradiation is a p53 transcription-dependent event and PUMA promotes Bax translocation both by directly interacting with Bax and by competitive binding to Bcl-XL in UV-induced apoptosis [11-12]. Furthermore, BimL is involved in Bax activation during UV irradiation-induced apoptosis [13-14]. These results indicated that UV-induced apoptosis is a highly complex process.

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During apoptosis, the mitochondrial network fragmented into smaller and more numerous mitochondria [15]. Drp1 and other proteins participate in regulating the fission of mitochondria. However, the precise role of mitochondrial fission during apoptosis needs to be further clarified.

In this study, we demonstrate that Drp1 translocates from cytosol to mitochondria and accumulates on mitochondria during UV-induced apoptosis. Furthermore, Drp1 regulates the transition from an interconnecting network to a punctiform mitochondrial phenotype, which may be contributed to cell dysfunction and UV-induced apoptosis.

2 Materials and Methods

2.1 Cell Culture

Human lung adenocarcinoma cells (ASTC-α-1) and HeLa cells were grown on 22-mm culture glasses, in Dulbecco’s modified Eagle’s medium (DMEM, Life Technologies, Inc.), supplemented with 15% fetal bovine serum, 50 units/mL penicillin, and 50 µg/mL streptomycin in 5% CO2, 95% air at 37°C in a humidified incubator.

2.2 Plasmids and Treatments

The pYFP-Drp1 was kindly supplied by Heidi M. McBride (Rodolfo Zunino et al., 2009) and pDsRed-mit was kindly supplied by Dr. Y. Gotoh (Tsuruta et al., 2002). Transfections were performed with Lipofectamine 2000 reagent (Invitrogen), according to the manufacturer’s protocol. The medium was replaced with fresh culture medium after 5 h. Cells were examined at 24–48 h after transfection. For UV treatment, medium was removed and saved. The cells were rinsed with phosphate-buffered saline (PBS) and irradiated, and the medium was restored. Unless otherwise specified, cells were exposed to UV irradiation at a fluence of 120 mJ/cm² and observed at the times indicated.

2.3 Time-lapse confocal fluorescence microscopy

YFP and DsRed fluorescence were monitored confocally using a commercial laser scanning microscope (LSM 510/ConfoCor 2) combination system (Zeiss, Jena, Germany) equipped with a Plan-Neofluar 40×/1.3 NA Oil DIC objective. Excitation wavelength and detection filter settings for each of the fluorescent indicator were as follows: YFP fluorescence was excited at 488 nm with an argon ion laser and emission was recorded through a 500–550 nm band pass filter. DsRed fluorescence was excited at 543 nm with a helium-neon laser and emitted light was recorded through a 560 nm long pass filter. For time-lapse imaging, culture dishes were mounted onto the microscope stage that was equipped with a temperature-controlled chamber (Zeiss, Jena, Germany).

2.4 FRAP Analysis

For fluorescence recovery after photobleaching (FRAP) analyses, the same 40× objective was used. HeLa cells were plated on 22 mm culture glasses. After 24 hours, cells were transfected with DsRed-Mit to label mitochondria. The RFP signal from mitochondria was bleached by using 100% of the 543- and 514-nm lines. Recovery was monitored by scanning with the 543-nm line every 5 s for 5 min. Quantification of recovery kinetics was performed by using the Zeiss LSM software.

2.5 Drp1-YFP Translocation Assay

To monitor Drp1-YFP translocation in living cells, ASTC-α-1 cells were cotransfected with Drp1-YFP and DsRed-Mit. Using an LSM 510 confocal microscope (Carl Zeiss), we imaged both the distribution pattern of Drp1-YFP and that of DsRed-Mit simultaneously during UV-induced apoptosis. Drp1 redistribution was assessed by the matching fluorescence of Drp1-YFP and DsRed-Mit emission. The cells exhibiting strong punctate staining of YFP, which overlapped with the distribution of DsRed, were counted as the cells with mitochondrial localized Drp1.
3 RESULTS

3.1 The Conversion of the Mitochondrial Network into a Punctiform Phenotype during UV-induced apoptosis.

To visualize mitochondria, ASTC-α-1 cells and HeLa cells were transiently transfected with pDsRed-mit for localizing mitochondria. 24-48 hours after transfection, cells were treated with UV (120 mJ/cm²) to induce apoptosis and visualized by confocal microscopy.

A.

B.
Figure 1. The Conversion of the Mitochondrial Network into a Punctiform Phenotype during UV-induced apoptosis. ASTC-α-1 (A) and HeLa (B, C) cells undergoing profound changes of their mitochondrial phenotype with UV (120mJ/cm²) treatment. In both cells, the characteristic tubular mitochondrial morphology disintegrates into numerous round fragments. Rapid recovery of fluorescence after photobleaching (B, C) is indicative of mitochondrial connectivity, while a failure to recover fluorescence is indicative of mitochondrial discontinuity or fragmentation. Bar=10μm.

3.2 Drp1-YFP predominantly locates to cytosol under physiological conditions

To visualize mitochondria, ASTC-α-1 and HeLa cells were transiently co-transfected with DsRed-mit and Drp1-YFP. 24-48 hours after transfection, cells were evaluated using laser confocal microscopy.

A.
Figure 2. Subcellular localization of Drp1-YFP in ASTC-α-1 and HeLa cells.

ASTC-α-1 cells (A) and HeLa cells (B) were co-transfected with DRP1-YFP and DsRed-mit. In healthy mammalian cells, Drp1 has primarily a cytosolic subcellular distribution. Bar=10µm.

3.3 Drp1 Translocates to the Mitochondria during UV-induced Apoptosis

A.

Fig. 3. (A) ASTC-α-1 cells were transfected with Drp1-YFP and DsRed-mit. In healthy ASTC-α-1 cells, most of the Drp1-YFP has a diffuse cytosolic distribution. During UV-induced apoptosis (120mJ/cm²), Drp1-YFP translocates from cytosol to mitochondria, indicating the enhancement of Drp1 mitochondrial accumulation. Bar=10µm.
**DISCUSSION**

In this study, we demonstrate that during UV-induced apoptosis the mitochondrial reticulum of mammalian cells disintegrates into multiple punctiform mitochondria. We find that Drp1, dynamin-related GTPase, is involved in the profound switch of mitochondrial phenotypes. Furthermore, we document the role of Drp1 in mitochondrial fission at steady state and during UV-induced apoptosis. Under apoptotic stimuli, Drp1 translocates to the mitochondria, and the typical reticular organization of mitochondria disintegrates into multiple small fragments. The apoptosis-associated translocation of Drp1 is consistent with the conclusion that Drp1 is involved in the transition from a tubular to a punctiform mitochondrial phenotype.

Previous reports indicate that Drp1 plays an important role in the progression of apoptosis \[16\]. However, mechanisms that are responsible for regulation of the mitochondrial fission during cell apoptosis are unknown. Furthermore, the mechanisms for recruitment of Drp1 to the mitochondria for spiral formation and mitochondrial fission remains unclear \[17\]. Although Drp1 is known to function as a mitochondrial fission enzyme, overexpression of Drp1 within cells does not significant increase mitochondrial fission \[18-19\], indicating that there are other limiting factors in the mitochondrial fission process. These factors may recruit cytosolic Drp1 to the outer mitochondrial membrane and/or activate the Drp1 GTPase, leading to mitochondrial fission. In future studies, we will focus on the mechanisms for the recruitment of Drp1 to mitochondria surface in response to UV-induced apoptosis.

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