Mitochondrial morphology and physiology are regulated by the processes of fusion and fission. Some forms of apoptosis are reported to be associated with mitochondrial fragmentation. We showed that overexpression of Fzo1A/B (rat) proteins involved in mitochondrial fusion, or silencing of Dnm1 (rat)/Drp1 (human) (a mitochondrial fission protein), increased elongated mitochondria in healthy cells. After apoptotic stimulation, these interventions inhibited mitochondrial fragmentation and cell death, suggesting that a process involved in mitochondrial fusion/fission might play a role in the regulation of apoptosis. Consistently, silencing of Fzo1A/B or Mfn1/2 (a human homolog of Fzo1A/B) led to an increase of shorter mitochondria and enhanced apoptotic death. Overexpression of Fzo1 inhibited cytochrome c release and activation of Bax/Bak, as assessed from conformational changes and oligomerization. Silencing of Mfn or Drp1 caused an increase or decrease of mitochondrial sensitivity to apoptotic stimulation, respectively. These results indicate that some of the proteins involved in mitochondrial fusion/fission modulate apoptotic cell death at the mitochondrial level.

Apoptosis plays an important role in various biological events in metazoans, including development and maintenance of tissue homeostasis. A family of cysteine proteases called caspases cleaves various cellular proteins and thus drives the process of apoptosis. It has been shown that the mitochondria play a pivotal role in apoptosis by releasing several apoptogenic molecules (such as cytochrome c, Smac/DIABLO, Omi/HtrA2, AIF, and endonuclease G) into the cytoplasm from the intermembrane space, after which these molecules activate downstream destruction programs, including the caspase cascade (1). The pro-apoptotic increase of mitochondrial membrane permeability is mainly regulated by members of the Bcl-2 family of proteins (2–4). Bcl-2 family consists of anti-apoptotic members such as Bcl-2 as well as pro-apoptotic members, including multidomain members such as Bax and Bak and numerous BH3-only proteins (e.g. Bid). BH3-only proteins, when activated, transmit apoptotic signals to the mitochondria to activate Bax and Bak (which act as a gateway), leading to mitochondrial membrane permeabilization (5, 6). The apoptotic mitochondrial membrane permeabilization may also be influenced by the mitochondrial physiological status, including respiration, lipid context, and fusion/fission (7).

Mitochondria are continuously fusing and dividing, processes that are thought to have a role in homeostasis. In Saccharomyces cerevisiae, fission of the outer mitochondrial membrane is driven by Dnm1p, Fis1p, and Mdv1p (8–10), whereas Mdm33p is involved in fission of the inner mitochondrial membrane (11). Dnm1p is a dynamin-related GTPase that is normally localized in the cytosol (8); it undergoes translocation and binds to the outer membrane via Fis1p during fission (9) and is thought to form a large oligomeric ring-like complex that pinches the outer mitochondrial membrane. Deletion of any of the fission genes blocks mitochondrial fission and produces a fused, interconnected mitochondrial network. Orthologs of Dnm1p have been characterized in Caenorhabditis elegans (DRP1) (12) as well as in mammals (Drp1) (13). Fzo1p and Ugo1p drive fusion of the outer mitochondrial membrane (14–16), whereas Mgm1p is involved in fusion of the inner mitochondrial membrane (17) in S. cerevisiae. Fzo1 was first characterized in Drosophila melanogaster and is a large GTPase that circumscribes the outer membrane and is essential for mitochondrial fusion (18). Mutations of the fzo gene in flies inhibit mitochondrial fusion during spermatogenesis, leading to a “fuzzy onion” morphology (18). A homolog of Fzo1 protein is found in mammals (e.g. rat Fzo1 and human Mfn) (19, 20). Defects of yeast fzo or the mammalian homologs of Fzo1 result in extensive fragmentation of the mitochondria and lead to cells being classed as the r0 phenotype, which lacks mitochondrial DNA (14).

Recent studies have suggested that the processes of mitochondrial fusion/fission are somehow involved in the regulation of apoptosis. During the early stage of apoptosis, the mitochondrial network is destroyed in mammalian cells (21–23). It has also been shown that overexpression of a dominant-negative Drp1 mutant (Drp1K38A) prevents apoptotic fragmentation of the mitochondrial network, as well as the occurrence of cytochrome c release, and apoptosis (21). Furthermore, silencing of Opa1 (a human homolog of Mgm1p) and overexpression of Fis1 both induce mitochondrial fragmentation, and reportedly also induce apoptosis (24, 25). However, the mechanisms by which fusion and fission processes are involved in apoptosis remain to be elucidated.

In the present study, we confirmed that mitochondrial fragmentation occurred during apoptosis in some cell lines. We also found that increasing or decreasing the expression of proteins involved in mitochondrial fusion (Fzo1/Mfn) relative to proteins involved in fission (Dnm1/Drp1) resulted in cells becoming more resistant or more sensitive to apoptotic stimulation, re-
respectively, by influencing the activation of Bax/Bak in the mitochondria.

**EXPERIMENTAL PROCEDURES**

**Chemicals and Antibodies**—Bismaleimidoethane was obtained from Pierce. The caspase inhibitor z-VAD-fmk\(^1\) was purchased from Peptide Inc. (Minoh, Japan).

An anti-Bax polyclonal antibody (N-20) and anti-Bak monoclonal antibody (Ab-1) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA) and Oncogene (Boston, MA), respectively. Anti-Drp1 and anti-Tom20 monoclonal antibodies were purchased from Transduction Laboratory (San Diego, CA). Anti-human Fas monoclonal antibody (CH-11), anti-cytochrome c (6H2 and 7H8) monoclonal antibodies, and goat anti-mouse Alexa Fluor 488 antibody were obtained from MBL (Nagoya, Japan), BD Pharmingen, and Molecular Probes (Eugene, OR), respectively. Anti-GAPDH antibody and anti-porin (voltage-dependent anion channel) monoclonal antibody were purchased from Chemicon (Temecula, CA) and Calbiochem, respectively.

**Cell Culture and DNA Transfection**—HeLa cells (a human cervical carcinoma cell line) and a derivative cell line with stable overexpression of human Bcl-2 (26) were maintained in RPMI1640 medium supplemented with 10% fetal bovine serum. Rat 1 cells (a rat fibroblast cell line) were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum. SV40 T antigen-transformed wild type (WT) and Bax/Bak double knockout MEFs were grown in modified Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum.

To visualize the mitochondria in living cells, DNA encoding Su9-DeR2 was used: this fusion construct was produced by insertion of the presence of mouse Fas, ATPase subunit 9 into the DeR2-N1 vector plasmid. DNA encoding truncated human Bid inserted into the pUC-CAGGS expression vector (27) and DNAs encoding rat Fzo1A/B inserted into the pCMV14 expression vector (Sigma) were also used. Cells (1 × 10\(^6\) cells) were transfected with plasmid DNA using the Amaza electroporation system according to the supplier’s protocol (kit V, program U-20). The efficiency of transfection was more than 75% as assessed by cotransfection of Su9-DeR2 DNA. All the siRNAs used were produced by Dharmacon Research, and the sequences were as follows (numbers in parentheses indicate nucleotide positions within the respective open reading frame): rat Fzo1A-siRNA (46–66), 5′-AAGCAACAUACAGGA-AUGGCU-3′; rat Fzo1B-siRNA (110–130), 5′-AAGCAACAUACAGGA-ACCCGG-3′; rat Dnm1-siRNA (96–116), 5′-AACUCAGAGCAGUGGA-ACCCGG-3′; human Drp1-siRNA (96–116), 5′-AACUCAGAGCAGUGGA-ACCCGG-3′; human Mfn1-siRNA (46–66), 5′-AAGGGGAUUAUCGCA-AUCUUU-3′; human Mfn2-siRNA (52–72), 5′-AAGAGACACAGGCU-GAGGG-3′; human Drp1-siRNA (96–116), 5′-AAGAGACACAGGCU-GAGGG-3′; and rat Fzo1A/B-siRNA (274–294), 5′-GCCUAGGCUA-GAGGCACC-3′. Cells (Rat 1 or HeLa cells) were transfected twice on alternate days with 10 μg of siRNA using the Amaza electroporation system.

**Analysis of Mitochondrial Morphology and Immunocytochemistry**—HeLa cells, Rat 1 cells, and MEFs were transiently transfected with Su9-DeR2, and mitochondrial morphology was analyzed under a fluorescence microscope (Zeiss; LSM510META). To determine the localization of Drp1, HeLa cells were grown in 8-well chamber slides and then fixed in 4% paraformaldehyde for 20 min at room temperature, permeobilized with 0.1% Triton X-100 for 20 min at room temperature, and blocked with 2% bovine serum albumin in PBS for 30 min at room temperature. Next, the cells were incubated with anti-Drp1 monoclonal antibody for 1 h at room temperature. After washing with PBS, the cells were stained with goat anti-mouse Alexa Fluor 488 antibody, washed twice with PBS, and examined under a confocal microscope.

**Subcellular Fractionation**—To assess Bax translocation and cytochrome c release, subcellular fractionation was performed using digitonin, as described previously (28). Briefly, after washing twice with PBS, cells were suspended in buffer (20 mM potassium HEPES, pH 7.4, 10 mM KCl, 1.5 mM MgCl\(_2\), 250 mM sucrose, 0.1 mM phenylmethysulfonyl fluoride, and 1 mM dithiothreitol), and treated with 30 μg/ml digitonin for 5 min at 4°C. Centrifugation was used to separate the cytosolic and organelar fractions, followed by lysis with radioimmune precipitation assay buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 0.5% deoxycholate, and 0.1% SDS). Isolation of the heavy membrane fraction enriched in mitochondria for measurement of Drp1 was done as described previously (29). Finally, aliquots of each fraction were subjected to Western blot analysis.

**Analysis of Protein Oligomerization**—Oligomerization of Bax was induced by gel filtration chromatography. HeLa cells were harvested, washed twice with PBS, and lysed with HNC buffer (25 mM sodium HEPES, pH 7.5, 300 mM NaCl, 2% CHAPS, 1 mM dithiothreitol, and 0.1 μM phenylmethylsulfonyl fluoride). After sonication and centrifugation (15,000 rpm for 15 min), the cell extract was collected and loaded onto a Superdex 200 HR 10/30 column (Amersham Biosciences) equilibrated in HNC buffer without dithiothreitol. Fractions of 96 μl were collected, and aliquots of each fraction were subjected to Western blotting.

To detect oligomerization of Bax, HeLa cells were treated with etoposide, harvested, and washed twice with PBS. Then the cells were resuspended in PBS, and Me2SO (control) or bismaleimidohexane was added at a final concentration of 0.1 mM. After incubation for 30 min at room temperature, the cells were collected and resuspended in protein sample buffer containing dithiothreitol to quench the cross-linking reaction.

**Analysis of Cell Death**—HeLa cells and Rat 1 cells were treated with 200 μM etoposide or 1 μM/ml anti-human Fas antibody (CH-11). Apoptotic cells were detected by examination of nuclear morphology after staining with Hoechst 33342 (1 μM).

**Permeabilized Cells**—HeLa cells grown in 10-well poly-l-lysine-coated glass slides, washed with isotonic buffer (20 mM potassium HEPES, pH 7.4, 1.5 mM MgCl\(_2\), 10 mM KCl, 250 mM sucrose), permeabilized with 20 μg/ml digitonin for 4 min at room temperature, and then washed with isotonic buffer. Permeabilized cells were incubated with recombinant human Bid protein for 10 min at 37 °C, washed with isotonic buffer, and fixed with 4% paraformaldehyde. Then the fixed cells were stained with anti-Bak (Ab-1) or anti-cytochrome c antibodies for 1 h at room temperature.

**RESULTS**

**Apoptotic Mitochondrial Fragmentation and Translocation of Drp1 Are Bcl-2-independent Processes**—Recent studies have shown that fragmentation of the mitochondrial network occurs during an early stage of apoptosis (21, 29, 30). To confirm this, HeLa cells were transiently transfected with DNA encoding mitochondria-targeting DeR2 and then treated with etoposide (an inhibitor of topoisomerase II). By transfection of cells with this plasmid, the mitochondria were specifically stained red irrespective of their membrane potential (data not shown). Whereas many mitochondria were elongated and created a network in healthy cells, the mitochondria became fragmented after addition of etoposide (Fig. 1, A and B), confirming previous observations (21, 29, 30). This change occurred at an early stage of apoptosis and was not affected by the caspase(s) inhibitor z-VAD-fmk or by Bcl-2 overexpression (Fig. 1, A and B), both of which almost completely inhibited apoptosis (Fig. 1F), suggesting that apoptotic mitochondrial fragmentation is a Bcl-2-insensitive and caspase-independent process. Apoptotic mitochondrial fragmentation was also observed when cells were subjected to other stimuli, such as anti-Fas and staurosporine, and also when other cell lines (COS7 and Rat1) were tested (data not shown). However, mouse embryonic fibroblast cells (MEFs) showed very limited mitochondrial fragmentation during etoposide-induced apoptosis (Fig. 1C).

To examine whether the mitochondrial fragmentation machinery was involved in apoptotic mitochondrial fragmentation, we examined the localization of a mitochondrial fission protein Drp1, which has been reported to accumulate in the mitochondria during fission as well as apoptosis in some cell lines (21, 30). Subcellular fractionation analysis revealed that etoposide induced translocation of a significant amount (approximately one-fifth) of Drp1 to the mitochondria (Fig. 1D). This was also confirmed by immunofluorescence study: after the cells were treated with etoposide, colocalization of Drp1 with Su9-DeR2 was increased as judged by significant increase of yellow color in the merged photos (Fig. 1E). Although etoposide-treated MEFs
showed little apoptotic mitochondrial fragmentation (Fig. 1C), there was significant translocation of Drp1 to the mitochondria as in HeLa cells (Fig. 1F). These results suggested that mitochondrial fragmentation varies between cell lines while a similar extent of Drp1 translocation to the mitochondria occurs during apoptosis, suggesting possible involvement of Drp1 and the mitochondrial fission apparatus in apoptotic events. Using this method, we also examined whether or not Bcl-2 had an influence on apoptotic translocation of Drp1. As shown in Fig. 1D, etoposide induced translocation of Drp1 that was not inhibited by Bcl-2, consistent with the lack of inhibition of apoptotic mitochondrial fragmentation (Fig. 1A), indicating that apoptotic translocation of Drp1 to the mitochondria occurs in a Bcl-2-independent manner.
Fzo1 Inhibits Etoposide-induced Apoptotic Mitochondrial Fragmentation and Various Types of Apoptosis—We next examined whether apoptosis was influenced by the inhibition of mitochondrial fission. Rat Fzo1 (corresponding to human Mfn), an essential protein for mitochondrial fusion, was introduced into HeLa cells. Because rats have two Fzo1 genes (Fzo1A and Fzo1B) that show 60% amino acid identity and are both essential for mitochondrial fusion (19), we introduced these two genes into HeLa cells concomitantly. Overexpression of Fzo1A/B increased elongated mitochondria (Fig. 2A). Not only etoposide-induced mitochondrial fragmentation, but also etoposide-induced apoptosis, was markedly delayed by Fzo1 overexpression (Fig. 2, A and B). Similar results were obtained when HeLa cells were treated with anti-Fas antibody (Fig. 2C), when Rat1 cells were treated with etoposide (Fig. 2D), and when MEFs were treated with etoposide (Fig. 2E). These results indicated that Fzo1A/B delays various types of apoptosis, possibly by inhibiting apoptotic mitochondrial fission.

Silencing of Fzo1 and Dnm1 (Proteins Involved in Mitochondrial Fusion and Fission) Alters Sensitivity of Cells to Apoptotic Stimuli—To determine whether alterations in the expression of genes involved in mitochondrial fusion/fission could influence the sensitivity of cells to apoptosis, we tested the effect of silencing rat Fzo1A/B and Dnm1 (a protein involved in fission) in Rat1 cells. As shown in Fig. 3A, Fzo1 and Dnm1 were almost completely eliminated by 48 h after the introduction of siRNA. After silencing of Fzo1, the filamentous mitochondrial network disappeared (Fig. 3B, middle panel), whereas silencing of Dnm1 resulted in the formation of elongated and interconnected mitochondria (Fig. 3B, right panel). When the cells were subsequently treated with etoposide, Fzo1-silenced cells were more sensitive to apoptosis than control cells, whereas Dnm1-silenced cells were more resistant to apoptosis (Fig. 3C). Similar results were obtained when HeLa cells were subjected to silencing of Mfn1/2 (the human homologs of Fzo1A/B, respectively) or Drp1 (the human homolog of Dnm1) (Fig. 3, D and E). Although cells with silencing of Fzo1A/B or Mfn1/2 showed...
**FIG. 3.** Mitochondrial fusion/fission balance affects sensitivity of cells to apoptotic stimuli. A–C, the effect of silencing of Fzo1 or Dnm1 on mitochondrial morphology and apoptosis of Rat1 cells. Rat1 cells were transfected twice on alternate days with either GFP siRNA, Fzo1A/B siRNA, or Dnm1 siRNA. A, at 48 h after the second transfection with siRNAs, expression of Fzo1A/B, Dnm1, and GAPDH (as a control) was analyzed by Western blotting. B, cells were treated as in A, and mitochondrial morphology (detection of Su9-DsRed2) was observed using a confocal fluorescence microscope. Representative images are shown. Magnified views are shown in insets. C, at 48 h after the second transfection, cells were treated with etoposide (200 μM). At the indicated times, apoptotic cells were counted by examining nuclear morphology. Closed squares: GFP siRNA; open circles: Fzo1A/B siRNA; open squares: Dnm1 siRNA. Data are shown as the mean ± S.D. (n = 3). D and E, the effect of silencing Mfn1/2 or Drp1 on mitochondrial morphology and apoptosis in HeLa cells. The same experiments as in B and C were performed using HeLa cells with either GFP siRNA, Mfn1/2 siRNA, or Drp1 siRNA. Closed squares: GFP siRNA; open circles: Mfn1/2 siRNA; open squares: Drp1 siRNA. Data are shown as the mean ± S.D. (n = 3).
fragmented mitochondria, death did not occur without apoptotic stimulation (Fig. 3, C and E). All these results indicated that modulation of genes involved in mitochondrial fusion and fission could significantly influence the sensitivity of cells to apoptosis.

**Fzo1 Inhibits Apoptosis by Delaying Cytosolic and Mitochondrial Cytochrome c Release**

Bax and Bak, which act as a gateway for various apoptotic signals at the mitochondria, are thought to exist as inactive forms in healthy cells, and various apoptotic stimuli may cause their activation through conformation changes and oligomerization, leading to cytochrome c release from the mitochondria (5, 6). Therefore, we first examined the effect of Fzo1 on apoptotic cytochrome c release in HeLa cells. As shown in Fig. 4 (A and B), Fzo1 delayed etoposide-induced and Fas-mediated release of cytochrome c, indicating that Fzo1 acted upstream of cytochrome c release. Next, we examined the effect of Fzo1 on etoposide-induced activation of Bax/Bak and found that oligomerization of both Bax and Bak was markedly delayed by Fzo1 expression (Fig. 4, C and D). Bax (but not Bak) is mainly localized in the cytoplasm of healthy cells and shows translocation to the mitochondria after apoptotic stimulation. Interestingly, overexpression of Fzo1 inhibited apoptotic mitochondrial localization of Bax (Fig. 4E), which might have led to
a delay in Bax activation. Taken together, these findings indicate that Fzo1 expression delayed the activation of Bax/Bak and thereby inhibited both cytochrome c release and apoptosis.

The Balance between Fusion and Fission Proteins Affects Mitochondrial Sensitivity to Death Stimuli—Changes in the levels of proteins involved in mitochondrial fusion/fission had an effect on apoptosis. Overexpression of Fzo1 inhibited apoptotic mitochondrial fragmentation and delayed Bax/Bak activation and cytochrome c release during apoptosis, raising the possibility that changes of the proteins involved in mitochondrial fusion/fission could alter mitochondrial sensitivity to apoptotic stimulation. To examine this issue, HeLa cells were transfected with siRNA for Mfn1/2 or Drp1, permeabilized with digitonin, and then incubated with recombinant Bid, which is a pro-apoptotic member of the Bcl-2 family and activates Bax/Bak (5, 6). Using this method, mitochondria in different states could be subjected to the same apoptotic stimulus, and the response was assessed by measuring cytochrome c release and Bak activation using a conformation-specific monoclonal antibody that only reacted activated Bak. Immunofluorescence microscopy demonstrated that recombinant Bid caused the activation of Bak in control cells but not in Bcl-2-overexpressing cells (Fig. 5, A and B). Because activation of Bak is cancelled by Bcl-2 in several cell lines, our in vitro system corresponded well with the cellular response. As shown in Fig. 5 (A and B), Mfn1/2-silenced cells largely had fragmented mitochondria and showed enhanced activation of Bak, whereas Drp1-silenced cells had elongated mitochondria and showed resistance to Bak activation. Consistent with these findings, Bid-induced cytochrome c release was accelerated by the silencing of Mfn1/2, whereas it was inhibited by silencing of Drp1 (Fig. 5, C and D). All these results suggested that the balance between mitochondrial fusion/fission proteins could affect mitochondrial sensitivity to apoptotic stimuli.

DISCUSSION

Here we showed that 1) mitochondrial fragmentation occurred during apoptosis and was accompanied by mitochondrial translocation of Drp1, whereas 2) changes in levels of mitochondrial fusion/fission proteins (Fzo1 (Mfn) and Dnm1 (Drp1)) affected the sensitivity of the mitochondria and cells to apoptotic stimulation. A higher fusion/fission protein ratio increased the resistance of mitochondria and cells to apoptotic stimulation, whereas a lower ratio had the opposite effect.
Because mitochondrial fragmentation was not observed in some cell lines, this process does not seem to be a prerequisite for apoptosis but rather to contribute to increased sensitivity to apoptotic stimuli.

How does the mitochondrial fusion/fission protein balance affect apoptosis? Although detailed mechanisms remain to be elucidated, one possibility is that, because fission and fusion of phospholipid membranes are regulated by these proteins, the mitochondrial lipid composition changes when the balance between fusion and fission protein is altered. Accumulating evidence indicates that changes of the phospholipid composition of the mitochondrial membrane can influence apoptosis. It has been reported that cardiolipin, which is a unique phospholipid in the mitochondria, is essential for the functioning of tBid during apoptosis (31) and for Bax-mediated permeabilization of liposomes (32), and that the intramitochondrial distribution of cardiolipin alters during apoptosis (33). Cardiolipin is localized in the inner mitochondrial membrane and accumulates at intermembrane contact sites (34), which have been suggested as a target for tBid and Bax (35, 36). Changes in the levels of mitochondrial fusion/fission proteins might alter the cardiolipin content of the mitochondrial membrane, thus affecting the sensitivity of the mitochondria and cells to apoptotic stimulation.

We also showed that Mfn/Fzo1-silenced cells, in which the mitochondria underwent fragmentation, did not die in the absence of additional apoptotic stimuli. These results were inconsistent with previous reports suggesting that silencing of Opa1 or overexpression of Fis1 caused both mitochondrial fragmentation and apoptosis (24, 25), so it is possible that Fis1 expression or Opa1 silencing not only provides a fission signal but also an apoptotic signal.

We also confirmed the previous observations (21, 29, 30) that Drp1 translocates from the cytosol to the mitochondria during apoptosis, although the mechanism regulating Drp1 translocation is unknown. It has been reported that a dominant negative mutant of Drp1 suppresses some forms of apoptosis (21), suggesting that the translocation of Drp1 to the mitochondria might be important for apoptosis by triggering mitochondrial fragmentation. We found that overexpression of Fzo1 significantly delayed the mitochondrial translocation of Drp1 (data not shown).

In this study, Bcl-2 did not inhibit either apoptotic mitochondrial fragmentation or translocation of Drp1 to the mitochondria (Fig. 1). We also found that apoptosis-induced transloca-
tion of Drp1 occurs equally in the absence of Bax and Bak (data not shown). Consistently, apoptotic mitochondrial fragmentation and Drp1 translocation were not observed when apoptosis was induced by overexpression of truncated Bid (tBid) (data not shown). Taken together, these results suggest that the Bcl-2 family of proteins does not seem to be involved in the processes of apoptotic mitochondrial fragmentation and Drp1 translocation, suggesting that various apoptotic stimuli (including etoposide, anti-Fas, etc.) not only activate Bax/Bak but also promote mitochondrial fragmentation that sensitizes the mitochondria to apoptotic signals.

Finally, it is interesting to note that several diseases are associated with mutations of genes involved in mitochondrial fusion/fission. Mutations of the *opa1* gene have been reported in autosomal dominant optic atrophy patients with degeneration of the retinal ganglion cells (37). Furthermore, it has been described that a mutation of the *mfn2* gene causes Charcot-Marie-Tooth neuropathy type 2A (38). It is possible that defects of mitochondrial fusion/fission might alter the susceptibility of cells to apoptotic death and, thus, cause these diseases.

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