

RESEARCH ARTICLE

Increased mitochondrial fission is critical for hypoxia-induced pancreatic beta cell death

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Abstract

Hypoxia-mediated pancreatic beta cell death is one of the main causes of pancreatic beta cell death, which leads to the loss of functional pancreatic beta cell mass and type 1 diabetes and type 2 diabetes. However, the molecular mechanisms that control life and death of pancreatic beta cells remain poorly understood. Here we showed that mitochondrial fission was strongly induced in pancreatic beta cells mainly due to an elevation of DRP1^{S616} phosphorylation through HIF-1 α activation and subsequent DRP1 mitochondrial translocation. Hypoxia-induced pancreatic beta cell death can be reversed by the inhibition of mitochondrial fission via DRP1 knockdown. We further demonstrated that hypoxia-induced mitochondrial fission untightened the cristae formation, which subsequently triggers mitochondrial cytochrome c release and consequent caspase activation. Moreover, treatment with mitochondrial division inhibitor-1 (Mdivi-1), a specific inhibitor of DRP1-mediated mitochondrial fission, significantly suppressed beta cell death in vitro, indicating a promising therapeutic strategy for treatment of diabetes. Taken together, our results reveal a crucial role for the DRP1-mediated mitochondrial fission in hypoxia-induced beta cell death, which provides a strong evidence for this process as drug target in diabetes treatment.

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Introduction

A fundamental challenge in treating diabetes is the identification of the molecular base that cause beta cell failure in response to environmental stress factors, including hypoxia. More and more studies support that pancreatic beta cells are heavily dependent on mitochondrial respiration and commonly sensitive to hypoxic stress due to their high consumption of oxygen during insulin secretion [1, 2]. Hypoxia-mediated cell death is still one of the main problems that must be solved for transplantation to be regarded as a reliable therapy [3]. However, the molecular mechanisms behind this are poorly understood.

Mitochondria are multifunctional and highly dynamic organelles, which are regulated by constant fusion and fission events [4]. Balanced fusion and fission is critical for appropriate numbers, morphology and activity of mitochondrial to satisfy the variable need of cells and

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adapt to the cellular environment [5]. To date, several core components of fusion and fission machinery have now been identified, including mitofusins (MFN1 and MFN2) and *optic atrophy 1* (OPA1) for mitochondrial fusion and dynamin-related protein 1 (DRP1), mitochondrial fission 1 protein (Fis1) and mitochondrial fission factor (MFF) for mitochondrial fission [6]. Moreover, recent studies have indicated that mitochondrial fission and fusion play a role in the regulation of cell apoptosis, showing that increased mitochondrial fusion suppresses apoptosis, whereas elevation in fission favors apoptosis [7–9]. However, we still do not know the role of mitochondrial fusion and fission in hypoxia-induced pancreatic beta cell death.

It is well known that cytochrome c release from mitochondria to cytosol is a critical step to cell death, which subsequently resulted in caspase activation and apoptotic cell death [10, 11]. Cristae are folded structures that greatly increase the total surface area of the inner membrane of mitochondria, providing more space for the series of compounds such as respiratory chain including cytochrome c [12, 13]. A previous study showed that OPA1-mediated mitochondrial fusion contributes to cristae reformation and cytochrome c release inhibition [14], implicating that mitochondrial dynamic-regulated cristae remodeling plays a critical role in cell apoptosis regulation.

Here we investigated the changes of mitochondrial morphology in pancreatic beta cells and their functional roles in the regulation of cell death and survival during hypoxia situations (1% O₂). Moreover, the underlying mechanisms and therapeutic application was systematically explored.

Materials and methods

Cell culture

Rat insulinoma cell line INS-1E, a gift from Dr. P. Maechler (University of Geneva, Switzerland), was cultured in RPMI-1640 media supplemented with 10% fetal bovine serum (Hyclone), 50 μmol/l β-mercaptoethanol, 1 mmol/l sodium pyruvate, 50 U/ml penicillin and 50 μg/ml streptomycin. For DRP1 silencing, INS-1E cells were transfected with siRNA against DRP1 (5' -CUACUCCUGAAAACAAC-3') or scrambled siRNA (5' -AATTCTCCGAACGTGTCA CGT-3') for 48 h using lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions.

Antibodies and reagents

The following primary antibodies were used in this study: anti-MFN1 (cat. #ab104585, Abcam), anti-MFN2 (cat. #ab101055, Abcam), anti-OPA1 (cat. #ab90857, Abcam), anti-DRP1 (cat. #ab56788, Abcam), anti-Fis1 (cat. #ab156865, Abcam), anti-MFF (cat. #ab125079, Abcam), anti-DRP1(S616) (cat. #4494, Cell Signaling), anti-DRP1(S637) (cat. #4867, Cell Signaling), anti-HIF-1α (cat. 610958, BD Biosciences), anti-CDAC (cat. #ab14734, Abcam), anti-β-actin (cat. TDY041, Beijing TDY BIOTEC) and horseradish peroxidase-conjugated anti-rabbit (cat. S004, Beijing TDY BIOTEC) and anti-mouse secondary antibody (cat. S001, Beijing TDY BIOTEC). The DRP1 inhibitor Mdivi-1 was purchased from Sigma-Aldrich (cat. M0199).

Transmission electron microscopy (TEM) for mitochondrial morphology analysis

For conventional TEM analysis, pancreatic beta cells were firstly fixed by glutaraldehyde. Then the cells were OsO₄ post-fixed, alcohol dehydrated, and embedded in araldite. After staining with uranyl acetate and lead citrate, sections were analyzed by using a Tecnai G2 electron microscope (FEI, Hillsboro, Oregon). In addition, imageJ software (NIH, Bethesda, MD) was used for mitochondrial length and cristae width analysis.

Mitochondrial morphology analysis by confocal microscopy

For fluorescence analysis of mitochondrial morphology by confocal microscopy, The fluorescent dye MitoTracker green FM (Molecular Probes, M7514) was used to monitor mitochondrial morphology in pancreatic beta INS-1E cells according to the manufacturer's instructions. Then cells were viewed and photographed with an Olympus FV 1000 laser-scanning confocal microscope (Olympus Corporation, Tokyo, Japan). The length of mitochondria was measured using the ImageJ software. A cell with fewer than 25% of the mitochondria visible in the cell had a length five times its width was judged to have fragmented mitochondria. A cell with greater than 75% of the mitochondria had a length five times its width highly was judged to have tubulated mitochondria interconnected if greater than 75% of the mitochondria had a length five times its width. Greater than 50 cells per group were blindly analyzed by three people.

Mitochondrial isolation

A cell mitochondria isolation kit (Beyotime, Nantong, China) was used for isolation of mitochondria from pancreatic beta INS-1E cells according to the manufacturer's instructions. Firstly, cells were washed twice with ice-cold PBS and resuspended in mitochondrial isolation buffer on ice for 20 min. Then, cells were centrifuged at 600 g for 10 min in 4°C before fully homogenized. The supernatant was centrifuged again in another tube at 11,000 g for 10 min in 4°C. At last, pellet was resuspended in mitochondria storage buffer.

Real-time PCR and western blot analysis

Total RNA was extracted with Trizol reagent (Invitrogen, Carlsbad, CA) and then reverse transcribed into cDNAs by using PrimeScript® RT Reagent Kit (Takara). The amplifications were carried out using the SYBR Green PCR Master Mix (Applied Biosystems). The primers used in this study were: MFN1 sense 5' -TGGCTAAGAAGGCGATTACTGC-3', and antisense, 5' -TCTCCGAGATAGCACCTCACC-3'; MFN2 sense 5' -CTCTCGATGCAACT-CTATCGT C-3', and antisense, 5' -TCCTGTACGTGTCTTCAAGGAA-3'; OPA1 sense 5' -TGTGAGGT CTGCCAGTCTTTA-3', and antisense, 5' -TGTCCTTAATTGGGGTCGTTG-3'; DRP1 sense 5' -GGAGACTCATCTTTGGTGAAGAG-3', and antisense, 5' -AAGGAGCCAGTCAAATTAT TGC-3'; Fis1 sense 5' -GTCCAAGAGCACGCAGTTTG-3', and antisense, 5' -ATGCCTTTA CGGATGTCATCATT-3'; MFF sense 5' -ACTGAAGGCATTAGTCAGCGA-3', and antisense, 5' -TCCTGCTACAACAATCCTCTCC-3'; GAPDH sense 5' -CGGAGTCAACGGATTTGGTC G-3', and antisense 5' -AGCCTTCTCCATGGTGGTGAAGAC-3', was used as control. The relative quantification in gene expression was determined by using the $2^{-\Delta\Delta C_t}$ method.

For western blot analysis, proteins were separated with 8% or 10% SDS-polyacrylamide gel electrophoresis and then transferred to a PVDF membrane. After blocked with 5% non-fat milk, the membranes were incubated with primary antibodies overnight at 4°C and HRP-conjugated secondary antibody for 2 h at 28°C. Blots were washed and detected by western maximum signal sensitivity kit (Pierce).

MTS assay

MTS assay was used for cell viability determination. Briefly, cells were collected and wash by PBS, and then incubated in 20ml of MTS (0.2%)-PMS (0.092%) solution for 2 h. Absorbance was detected at 490 nm. Each sample was analyzed in triplicate.

Apoptosis assays

Abcam’s Annexin V-FITC Apoptosis Staining/Detection Kit was used for cell apoptosis analysis by flow cytometry. Briefly, pancreatic beta INS-1E cells were collected and resuspended by 500 µl binding buffer with 5 µl ANXA5-FITC and 5 µl PI. Then, cells were incubated for 15 min at room temperature in the dark and analyzed with a flow cytometer (Beckman, Fullerton, CA). TUNEL assay (Roche, 11684795910) was also used for analysis of apoptosis in pancreatic beta cells following the manufacturer’s instructions. Images were obtained by a fluorescence microscope.

Statistical analysis

The data represent mean ± SD. All experiments were repeated 3 times. SPSS 16.0 software was used for all statistical analyses. Comparisons between two groups were performed with an unpaired two-tailed Student’s t test and multiple group comparisons were performed by unpaired one-way ANOVA followed by post hoc Turkey’s test. Differences of a *P*-value < 0.05 were considered as statistically significant.

Results

Hypoxia induces mitochondrial fragmentation in pancreatic beta cells

To investigate the impact of hypoxia on the mitochondrial dynamics in pancreatic beta cells, the alterations of mitochondrial morphology were examined by staining with mitochondrial dye, MitoTracker Green. Fluorescence microscopy analysis showed that hypoxia (1% or 3% O₂) treatment resulted in a dramatic increase of mitochondrial fragmentation in pancreatic beta INS-1E cells, with a higher increase at 1% O₂ than at 3% O₂ of mitochondrial, indicating an adose-dependent manner (Fig 1A and 1B). Transmission electron microscopy (TEM) further revealed a significantly lower average mitochondrial length in pancreatic beta INS-1E cells with hypoxia (1% or 3% O₂) treatment compared with those under normoxia (Fig 1C and 1D).

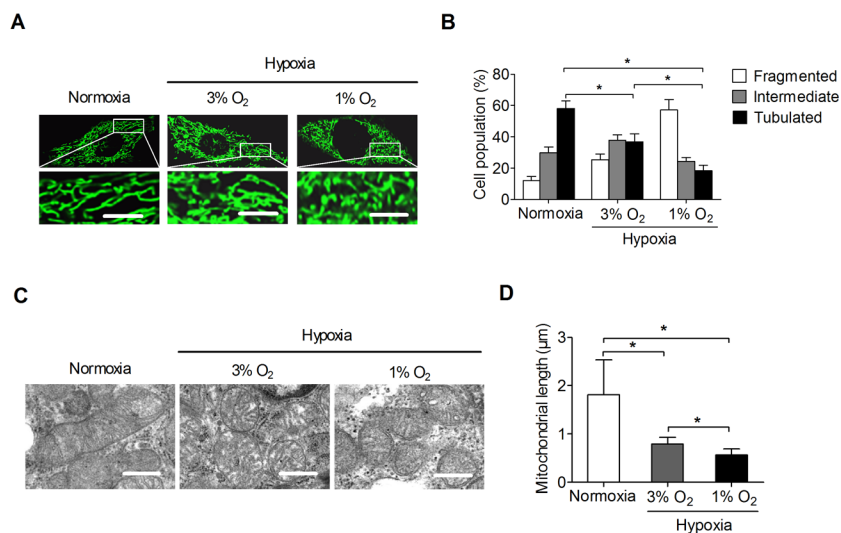


Fig 1. Hypoxia induces mitochondrial fragmentation in pancreatic beta INS-1E cells. A. Representative confocal microscope images of the mitochondrial in pancreatic beta INS-1E cells after treatment with hypoxia at different oxygen levels (3% or 1% O₂ for 24 h) as indicated. Scale bars, 10 µm. B. The proportion of pancreatic beta INS-1E cells with tubulated, intermediate, and fragmented mitochondria was quantified. C. Representative transmission electron microscopy (TEM) images of the mitochondrial in INS-1E cells treated as indicated. Scale bars, 0.5 µm. D. Image J was used for mitochondrial length quantification in INS-1E cells treated as indicated. All experiments were repeated three times. **P* < 0.05.

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HIF-1 α activation and subsequently DRP1(S616) phosphorylation is involved in hypoxia-induced mitochondrial fragmentation in pancreatic beta cells

To explore the underlying mechanism of hypoxia-induced mitochondrial fragmentation in pancreatic beta cells, we analyzed the expression levels of core mitochondrial dynamic mediators, including MFN1, MFN2 and OPA1 for mitochondrial fusion and DRP1, Fis1 and MFF for mitochondrial fission under hypoxia conditions by RT-PCR and western blot analysis. Surprisingly, no significant expression changes of MFN1, MFN2, OPA1, DRP1, Fis1 and MFF were observed at either the mRNA or protein level in pancreatic beta INS-1E cells with hypoxia treatment as compared to those under normoxia (Fig 2A and 2B). It is well known that hypoxia-

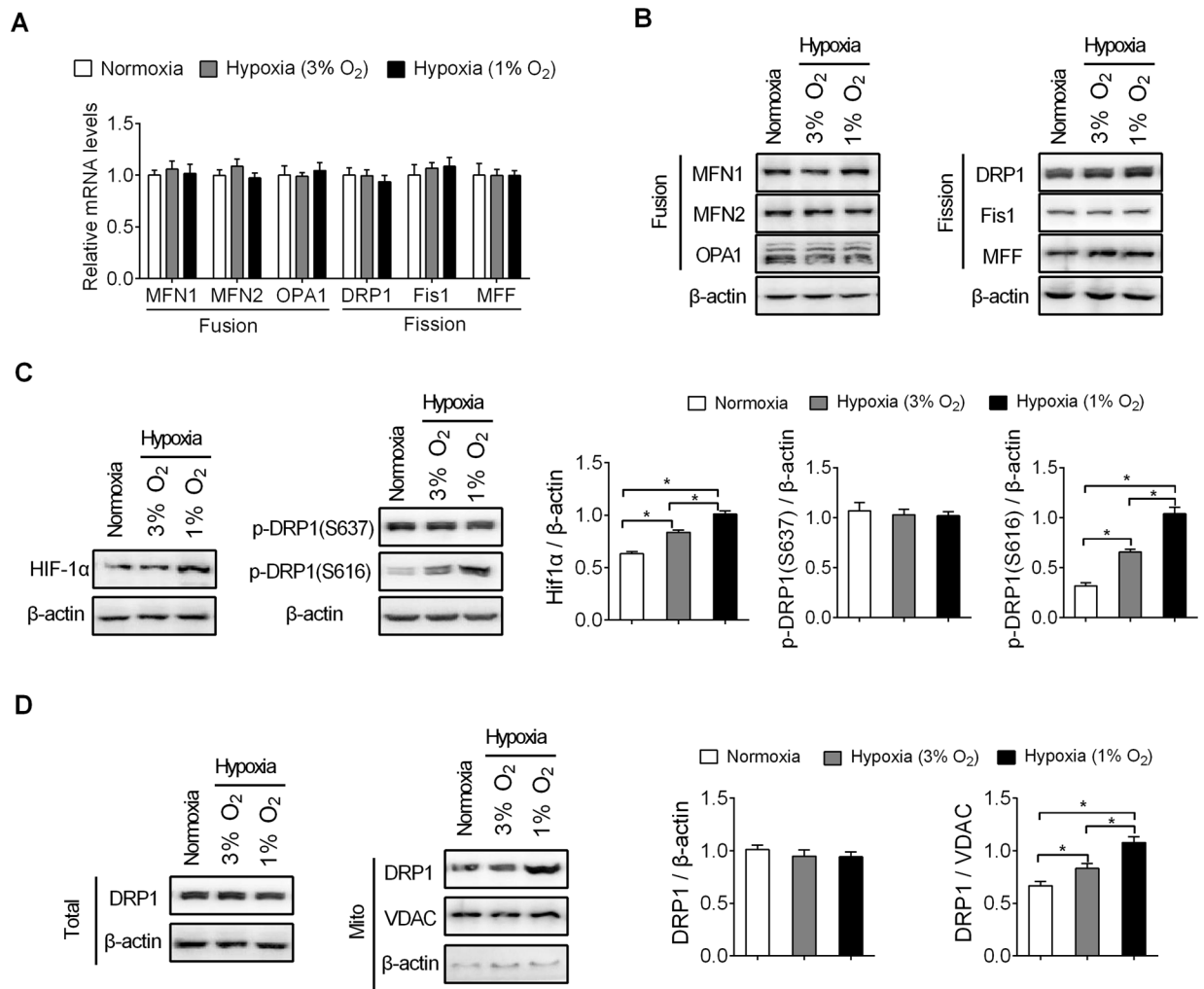


Fig 2. HIF-1 α activation and subsequently DRP1(S616) phosphorylation is involved in hypoxia-induced mitochondrial fragmentation in pancreatic beta cells. A-B. Expression levels of MFN1, MFN2, OPA1, DRP1, Fis1, and MFF were detected by RT-PCR (A) and western blot (B) analysis in pancreatic beta INS-1E cells with treatment as indicated. (C) Top: Levels of HIF-1 α and phosphorylated DRP1 (S637) or (S616) were detected by western blot analysis in pancreatic beta INS-1E cells with treatment as indicated. Bottom: Western blot scanning densitometry for three independent experiments. Blots were probed for β -actin to ensure equal protein loading. *P<0.05. (D) Top: Levels of DRP1 in total lysates and mitochondrial fraction were examined by western blot analysis in pancreatic beta INS-1E cells with treatment as indicated. VDAC serves as loading controls. Mito: mitochondria. Bottom: Western blot scanning densitometry for three independent experiments. Blots were probed for β -actin or VDAC respectively to ensure equal protein loading. *P<0.05. All experiments were repeated three times.

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inducible transcription factor (HIF-1 α) is a major regulator of cellular adaptation to low oxygen stress. A previous study has shown HIF-1 α activation can promote mitochondrial fission by phosphorylation of DRP1 at Serine-616 [15], a critical post-translational modification of DRP1 for its translocation from cytoplasm to mitochondrial. Therefore, we hypothesized that hypoxia-mediated HIF-1 α activation and subsequently DRP1(S616) phosphorylation may be involved in the regulation of mitochondrial fragmentation by hypoxia in pancreatic beta cells. As shown in Fig 2C, a dramatic increase of both HIF-1 α and DRP1(S616) phosphorylation were observed in pancreatic beta INS-1E cells upon hypoxia treatment. While no significant change in the phosphorylation of DRP1 at Serine-637, an inhibitory modification for fission activity of DRP1, was detected upon treatment with hypoxia. Moreover, the level of DRP1 in the mitochondrial fractions was significantly increased upon hypoxia treatment, further confirming the importance of DRP1(S616) phosphorylation in hypoxia-induced mitochondrial fragmentation of pancreatic beta cells (Fig 2D).

Mitochondrial fragmentation is involved in pancreatic beta cell death in hypoxia conditions

To determine the biological effects of hypoxia-induced mitochondrial fragmentation on pancreatic beta cells, cell apoptosis was assessed in pancreatic beta INS-1E cells with inhibited mitochondrial fission by DRP1 knockdown under hypoxia. As shown in Fig 3A, hypoxia promoted phosphorylation of DRP1(S616) can be attenuated by DRP1 knockdown. In addition, hypoxia significantly induced cell apoptosis of pancreatic beta INS-1E cells when compared with those under normoxia. Whereas inhibition of the mitochondrial fission by knockdown of DRP1 significantly restored hypoxia-induced apoptosis (Fig 3B), indicating that DRP1(S616) phosphorylation-mediated mitochondrial fragmentation is an upstream factor for pancreatic beta cell death during hypoxia conditions. Furthermore, release of cytochrome c from mitochondria into cytosol, and cleavage of caspase 3 and caspase 9 were significantly induced by hypoxia treatment, whereas all of them were remarkably inhibited when DRP1 was knocked-down in pancreatic beta INS-1E cells (Fig 3C and 3D). Moreover, a marked increase of positive TUNEL (transferase-mediated dUTP nick-end labeling) staining was observed in pancreatic beta cells under hypoxia conditions. In contrast, the pro-apoptotic effects of hypoxia treatment were significantly reversed by inhibition of mitochondrial fission by DRP1 knockdown (Fig 3E). Collectively, these results indicate that mitochondrial fragmentation plays an essential role in promoting hypoxia-induced pancreatic beta cell death, and inhibition of mitochondrial fission by DRP1 knockdown could protect pancreatic beta cell against hypoxia-induced cell death.

Hypoxia-induced mitochondrial fragmentation facilitates cristae remodeling in pancreatic beta cells

A previous study has shown that OPA1-mediated mitochondrial fusion promotes cristae reformation and inhibits cytochrome c release [14], implying a critical role for cristae remodeling in cell apoptosis regulation. We thus examined the effect of hypoxia-induced mitochondrial fragmentation on the structure remodeling of cristae by using transmission electron microscopy (TEM). Representative transmission electron microscopy (TEM) images in Fig 4A showed that hypoxia significantly induced mitochondrial fragmentation in pancreatic beta cells, while DRP1 knockdown clearly reversed hypoxia-induced mitochondrial fragmentation (Fig 4B). Moreover, pancreatic beta cells with hypoxia treatment showed a significant increase of cristae width (Fig 4C) and reduction of cristae density, all of which could be reversed by DRP1 knockdown (Fig 4D). These results collectively indicate that mitochondrial fragmentation-mediated cristae remodeling should be, at least in part, involved in hypoxia-induced pancreatic beta cell death.

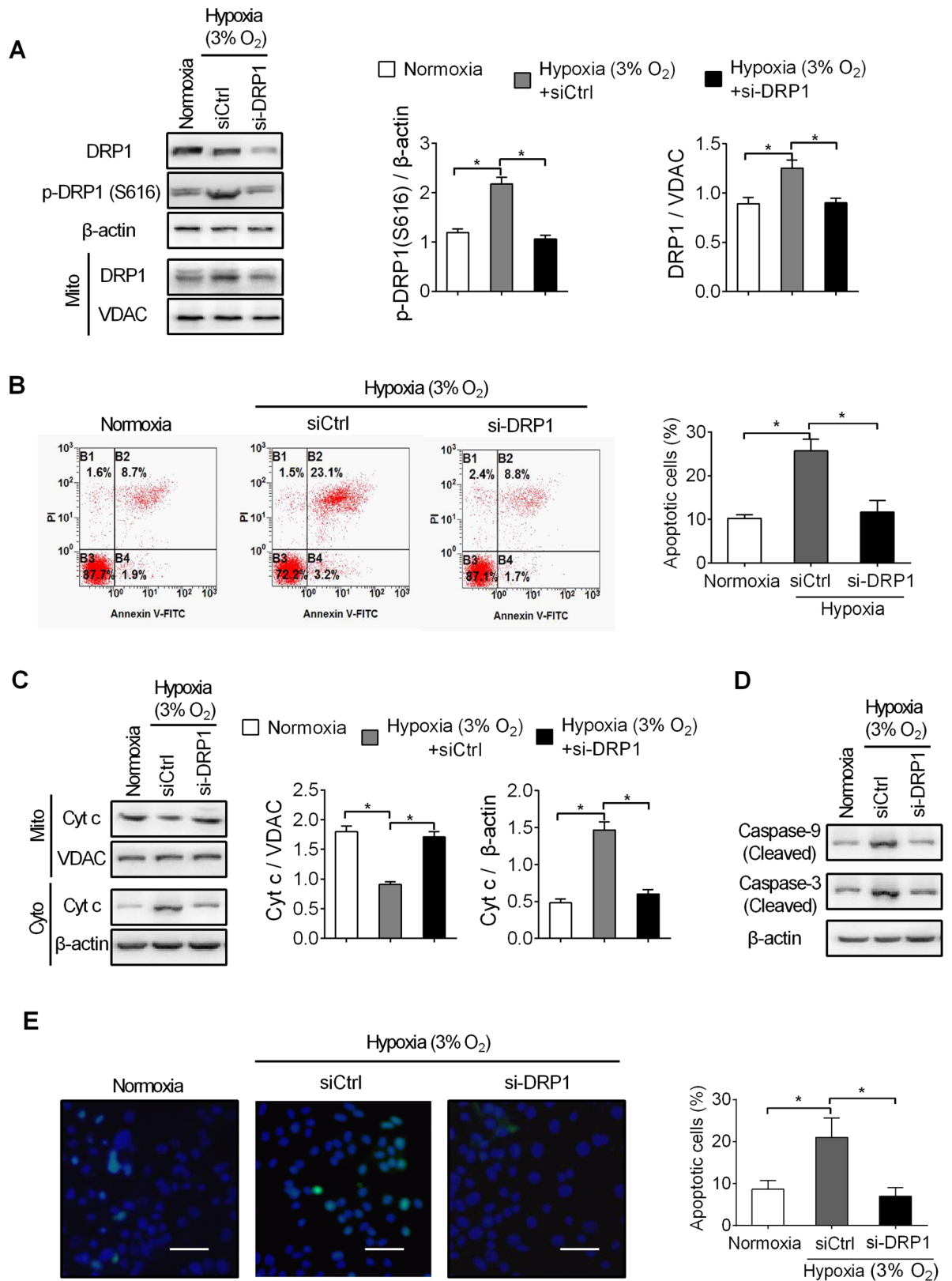


Fig 3. Mitochondrial fragmentation is involved in pancreatic beta cell death in hypoxia conditions. A. Detection of DRP1 (S616) and mitochondrial-fractional DRP1 by western blot analysis in pancreatic beta INS-1E cells with treatment as indicated. B. Detection of apoptosis

by flow cytometry in pancreatic beta INS-1E cells with treatment as indicated. C. Western blot analysis for levels of cytochrome c in pancreatic beta INS-1E cells with treatment as indicated. β -actin and VDAC were used as loading controls for cytoplasm and mitochondria, respectively. Cyto, cytoplasm; Mito, mitochondrial. D. Western blot analysis for levels of cleaved Caspase-9 and Caspase-3 in pancreatic beta INS-1E cells with treatment as indicated. E. TUNEL staining in pancreatic beta INS-1E cells from type 2 diabetes animal models. Blue: Hoechst 33342; Green: TUNEL positive nucleus. All experiments were repeated three times. * $P < 0.05$.

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Mdivi-1 exhibits a therapeutic effect on hypoxia-induced cell death of pancreatic beta cells

Mdivi-1 is a novel small-molecule inhibitor of mitochondrial fission that specifically targets Drp1. To explore the protective role of Mdivi-1 in hypoxia-induced pancreatic beta cell death, the effect of Mdivi-1 on mitochondrial fission and cell survival were firstly investigated. Our results showed that treatment with Mdivi-1 significantly inhibited mitochondrial fragmentation and clearly increased the viability of pancreatic beta INS-1E cells under hypoxia conditions (Fig 5A and 5B). Flow cytometry analysis further demonstrated that Mdivi-1 treatment significantly inhibited apoptosis of pancreatic beta INS-1E cells under hypoxia conditions (Fig 5C). Consistently, significantly fewer positive TUNEL staining cells were detected in Mdivi-1 treated pancreatic beta cells under hypoxia conditions compared with control by TUNEL assay (Fig 5D). Given the relation between hypoxia and OPA1, we determined the impact of Mdivi-1

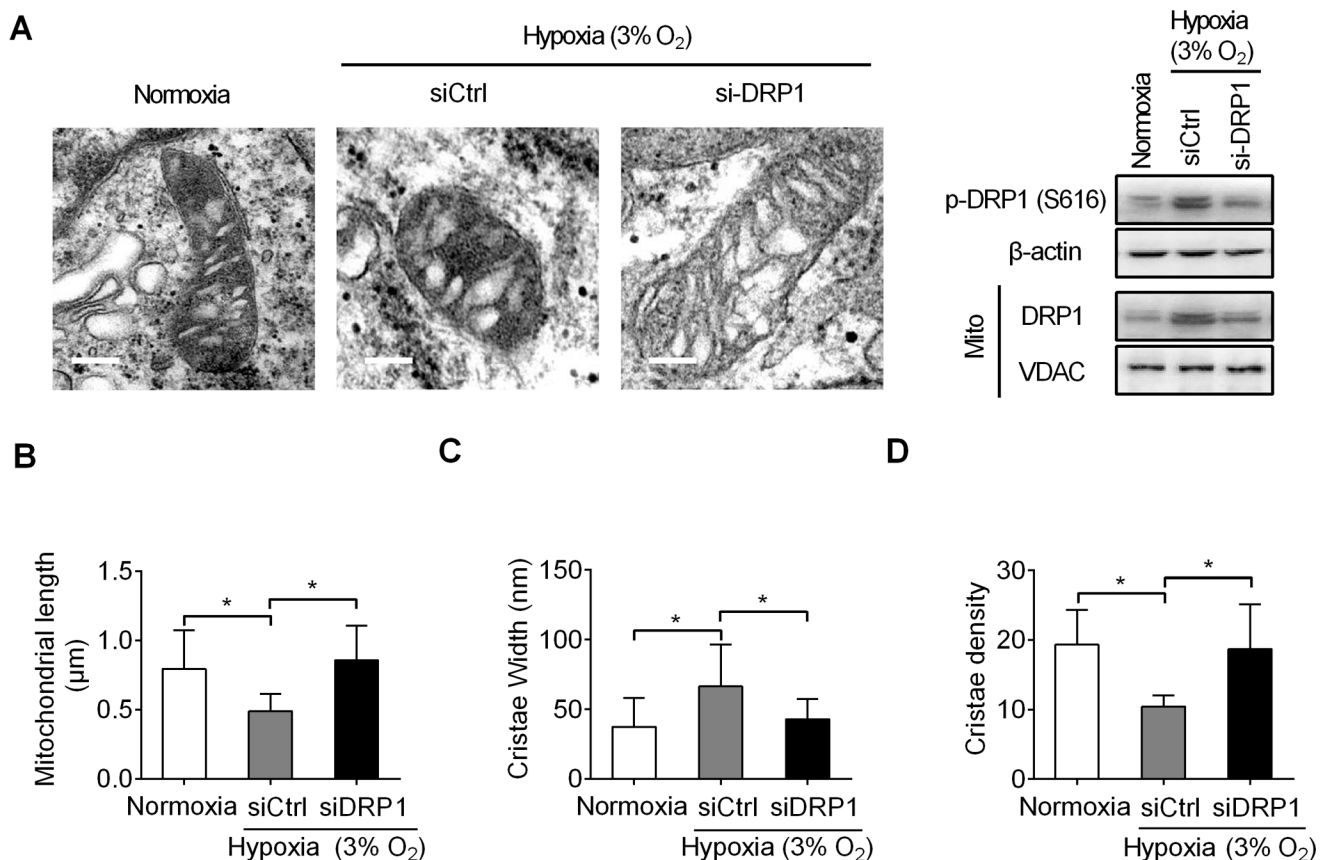


Fig 4. Hypoxia-induced mitochondrial fragmentation facilitates cristae remodeling in pancreatic beta cells. A. Representative transmission electron microscopy (TEM) images of mitochondria in pancreatic beta INS-1E cells treated as indicated. Scale bars, 0.1 μ m. B-D. Mitochondrial length (B), cristae width (C) and cristae density (number/mitochondrial length) (D) were quantitatively analyzed in pancreatic beta INS-1E cells treated as indicated. Over twenty mitochondria were randomly selected for quantitative analysis in each treatment group. All experiments were repeated three times. * $P < 0.05$.

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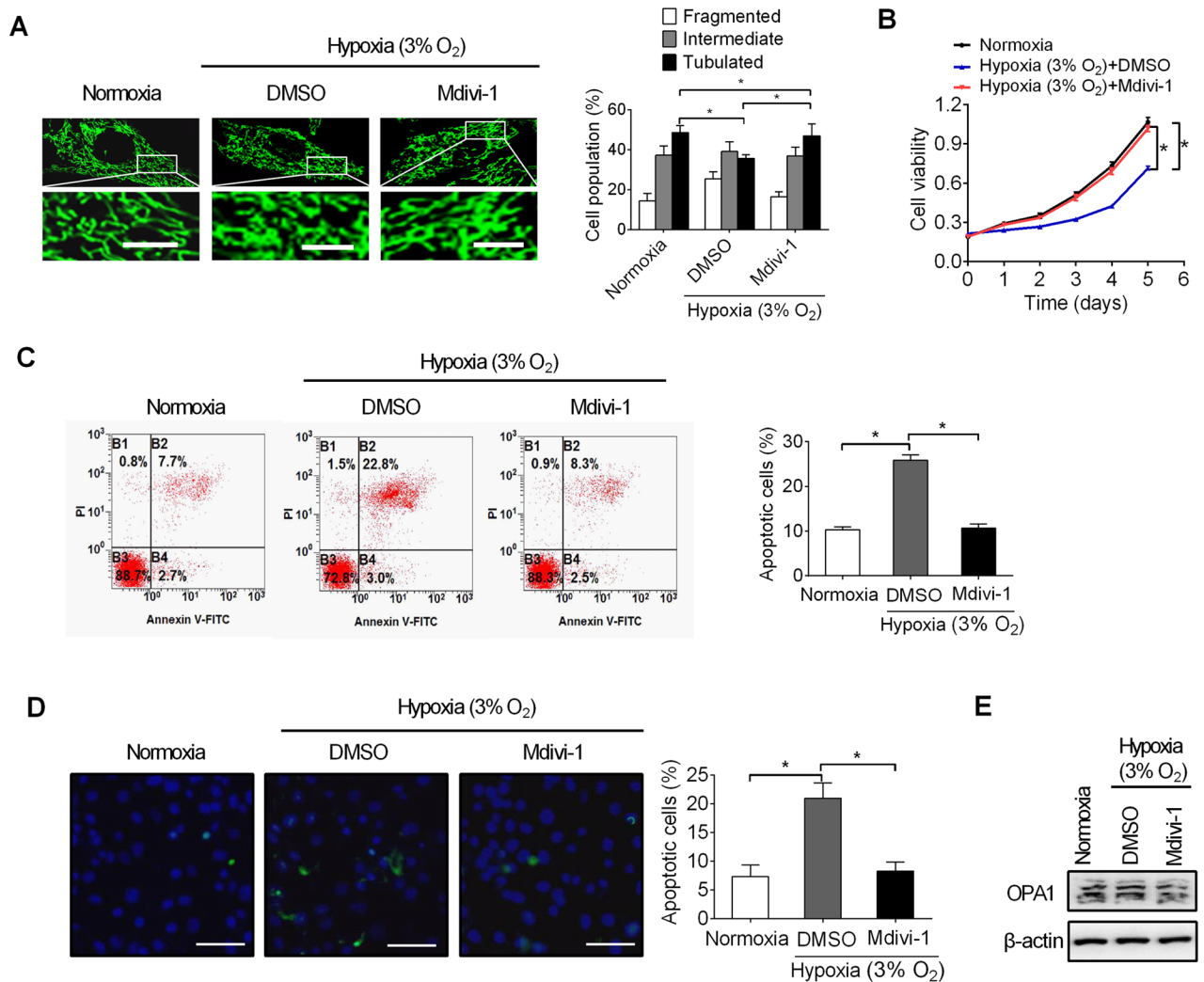


Fig 5. Mdivi-1 exhibits a therapeutic effect on hypoxia-induced cell death of pancreatic beta cells. **A.** Mitochondrial morphology analysis by confocal microscopy in pancreatic beta INS-1E cells treated with Mdivi-1 (50 μM) or DMSO for 12 h as indicated. Scale bars, 10 μm. **B.** Cell viability analysis by MTS assay in pancreatic beta INS-1E cells treated with Mdivi-1 or DMSO as indicated. **C.** Detection of apoptosis by flow cytometry in pancreatic beta INS-1E cells treated with Mdivi-1 or DMSO as indicated. **D.** TUNEL staining in pancreatic beta INS-1E cells treated with Mdivi-1 or DMSO as indicated. Scale bars, 50 μm. All experiments were repeated three times. * $P < 0.05$.

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treatment on the short and long forms of Opa1 in pancreatic beta INS-1E cells. As shown in Fig 5E, Mdivi-1 treatment did not notably affect the short and long forms of Opa1, suggesting that the protective role of Mdivi-1 in hypoxia-induced pancreatic beta cell death is not mediated by regulation of OPA1. These results collectively indicate that inhibition of mitochondrial fission by the Mdivi-1 is a promising therapeutic strategy for treatment of diabetes.

Discussion

The increasing evidence supports that hypoxia-mediated cell death is one of the main causes of pancreatic beta cell death, which leads to loss of functional pancreatic beta cell mass and thus type 1 and type 2 diabetes. However, the functional role of mitochondrial dynamics of fusion and fission, a major cellular stress response, in this process is still unclear. In the present study, we demonstrated that mitochondrial fragmentation is involved in hypoxia-induced

pancreatic beta cell death possibly by facilitating cristae remodeling. More importantly, Drp1 knockdown or Mdivi-1 treatment significantly suppressed hypoxia-induced pancreatic beta cell death, which strongly indicates a promising therapeutic strategy for treatment of diabetes.

Recently, cumulative evidence has indicated that mitochondrial dynamics of fusion and fission play a critical role in response to stimuli in the cellular environment. In this study, we observed that hypoxia treatment significantly increased mitochondrial fragmentation in pancreatic beta cells. Similar results were also obtained in other tissue types from several previous studies. T.H. Sanderson et al. have shown that hypoxia induces excessive fragmentation of mitochondria in isolated primary rat neurons [16]. In addition, Khushbu Jain et al. have also demonstrated that hypoxia induces alteration of mitochondrial morphology characterized by excessive mitochondrial fragmentation (fission) and decreased mitochondrial fusion in rat brain hippocampus [17]. Moreover, Wei Zuo et al. indicated that hypoxic conditions can lead to mitochondrial fission and the removal of dysfunctional mitochondria by autophagy in vascular endothelial cells [18]. Collectively, these results imply the importance of mitochondrial fragmentation in control of pancreatic beta cell death and survival during hypoxia conditions.

Hypoxia-inducible transcription factor (HIF-1 α) plays an essential role in cellular adaptation to low oxygen stress. Glenn Marsboom et al. have shown that HIF-1 α activation in human pulmonary arterial hypertension (PAH) leads to mitochondrial fission by cyclin B1/CDK1-dependent phosphorylation of DRP1 at Serine-616. Consistently, our present study also shows that hypoxia-mediated HIF-1 α activation clearly promotes DRP1 phosphorylation at Ser616 and subsequent mitochondrial fragmentation in pancreatic beta cells. However, we cannot entirely rule out the possibility of other kinases that may be involved in this process. Jennifer A. Kashatus et al. have shown that Erk2, another main cellular response to hypoxia, phosphorylates DRP1 on Serine 616 to promote mitochondrial fission in tumor cells [19]. Therefore, whether HIF-1 α acts alone or works together with other kinases to promote DRP1 S616 phosphorylation in pancreatic beta cells during hypoxia still needs further investigation. In addition, a previous study has shown that hypoxia can impact the 1 cleavage of fusion protein Opa1 in HEK293T and HeLa cells [20]. However, we demonstrated that hypoxia induces mitochondrial fragmentation of pancreatic beta cells not through expressing changes of any key mitochondrial dynamic regulators, but through a more rapid post-translational regulatory mechanism. These contradictions may be explained by the fact that the mitochondrial dynamic regulation under hypoxia conditions is very complicated and diverse regulatory mechanisms exist in different cell types. Cytochrome c is present in the cristae within the mitochondria and its release from the mitochondria is fatal to the initiation of caspase cascade and apoptosis. In addition, several studies have shown that mitochondrial dynamics of fission and fusion play a critical role in cristae reformation and the pro-apoptotic status of mitochondria. Tatiana Varanita et al. have reported that mitochondrial fusion protein OPA1-dependent cristae remodeling regulates the response of multiple tissues to apoptotic stimuli through inhibiting cytochrome c release from mitochondria [14]. In addition, both Luca Scorrano et al. and Ryuji Yamaguchi et al. have shown that mitochondrial fragmentation-mediated mitochondrial cristae remodeling through widening of cristae junctions are required for the release of cytochrome c across the outer mitochondrial membrane during apoptosis [21, 22]. Moreover, Otera et al. reported that Drp1-dependent mitochondrial fission regulates cristae remodeling of HeLa cells during intrinsic apoptosis [23]. Consistently, our study also showed that Drp1-mediated mitochondrial fission and cristae remodeling play an important role in hypoxia-induced pancreatic beta cell death. Moreover, we found that, under hypoxia conditions, DRP1(S616) phosphorylation-mediated mitochondrial fission was essential for pancreatic beta cell death, which is supported by a previous study in MEF cells demonstrating that mitochondria are fragmented to facilitate

cytochrome c release and cell death during apoptotic stimuli [24]. In contrast, stress conditions such as starvation, UV radiation and oxidation stress often induce mitochondrial elongation and hyperfusion, which play a protective role for cell survival under those stress conditions. These contradictions may be explained by the fact that mitochondrial dynamic regulation is very complicated and diverse regulatory mechanisms may exist in different cell types under different environmental stresses.

Mdivi-1 has been well accepted to be a small-molecule inhibitor of mitochondrial fission that specifically targets Drp1. In addition, due to its safety and protective benefits shown in vivo, Mdivi-1 has been shown to represent therapeutics for neurodegenerative diseases and human cancers [25, 26]. However, the ability of mdivi-1 to inhibit Drp1 and impact mitochondrial fission has recently been challenged. Bordt et al did not find any effects of mdivi-1 treatment on mitochondrial morphology in mammalian cells, whereas they found mdivi-1 could inhibit complex I of the electron transport chain and thus exert antioxidant effects. Accordingly, we still cannot rule out the possibility that anti-oxidant effect may also be involved in the protection of Mdivi-1 in hypoxia-induced pancreatic beta cell death, which still needs further investigation.

In summary, our data demonstrate that hypoxia treatment results in a dramatic mitochondrial fragmentation in pancreatic beta cells, which is mediated by HIF-1 α activation and subsequent DRP1S616 phosphorylation. Moreover, mitochondrial fragmentation-mediated cristae remodeling play an important role in hypoxia-induced pancreatic beta cell death, which could be reversed either by DRP1 knockdown or Mdivi-1 treatment, strongly suggesting a promising therapeutic strategy for diabetes treatment.

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Supervision: Yi Lv.

Writing – original draft: Da Zhang.

Writing – review & editing: Yi Lv.

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