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ORIGINAL RESEARCH

The Role of Placental MFF-Mediated Mitochondrial Fission in Gestational Diabetes Mellitus

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Introduction: Gestational diabetes mellitus (GDM) refers to hyperglycemia first recognized during pregnancy, characterized by decreased insulin sensitivity and impaired glucose metabolism. Dynamic fusion and fission processes within mitochondria play critical roles in maintaining glucose metabolism homeostasis. Given the fundamental role of mitochondrial fission factor (MFF) in mitochondrial fission, the intention of this study was to investigate mitochondrial dynamics in the placentae of GDM patients and explore the role of MFF in the etiopathogenesis and progression of GDM through the modulation of glucose metabolism and insulin resistance.

Methods: 40 Placental tissues were obtained from pregnant women undergoing cesarean section with GDM (n=20) and those with normoglycemia (n=20). To mimic the intrauterine high glucose environment, immortalized human-derived first-trimester extravillous trophoblast cells HTR8/SVneo were used and treated in a high glucose environment. Immunofluorescence was utilized to analyze MFF expression in placental tissues and mitochondrial length in HTR8/SVneo cells. The expression levels of glucose transporters (GLUTs) and other pivotal proteins involved in mitochondrial dynamics and the insulin signaling pathway, were assessed by Western blotting. Additionally, cellular glucose uptake capacity was determined using a glucose assay kit.

Results: MFF expression was greater in the GDM group than in the normoglycemic group. In a high-glucose environment, the expression of fusion-related proteins OPA1, MFN1 and MFN2 decreased while the expression of DRP1 and MFF increased, indicating that the mitochondrial dynamics of trophoblast cells shift toward fission. Elevated mitochondrial fission hinders the insulin signaling pathway, resulting in a reduction in glucose uptake by HTR8/SVneo cells and a concurrent decrease in GLUT4 expression.

Discussion: Our study demonstrates that MFF-mediated mitochondrial fission inhibits insulin sensitivity and upregulates glucose transport in GDM, which is related to offspring exposure to a hyperglycemic intrauterine environment. These results provide a novel therapeutic target for addressing GDM that may mitigate unfavorable pregnancy outcomes.

Keywords: MFF, insulin sensitivity, insulin resistance, gestational diabetes mellitus

Introduction

Gestational diabetes mellitus (GDM), defined as a first onset of hyperglycemia in the duration of pregnancy, is among the foremost complications that affects pregnant women.[1] Globally, the prevalence of GDM has exhibited a consistent increase, ranging from 10% to 100% over recent decades.[2] A meta-analysis has meticulously investigated the global standardized prevalence of GDM, revealing it to be 14.0%, with the highest standardized prevalence recorded in the Middle East and North Africa (27.6%) and Southeast Asia (20.8%).[3]

In certain studies, overweight, obesity[4] and advanced gestational age[5] are high risk factor of GDM. The repercussions of GDM include a spectrum of adverse pregnancy outcomes, including preeclampsia,[6] unanticipated cesarean deliveries,[7] and shoulder dystocia.[8] Over the long term, women afflicted with GDM face increased susceptibility to diabetes and

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cardiovascular disease. In addition, obesity and advanced age are aggravating factors to raise the incidence of these adverse outcomes.[9–11] Moreover, the exposure of offspring to hyperglycemic conditions in utero can result in an increased risk of obesity, cardiovascular disease, and metabolic syndrome.[12] Therefore, GDM plays a crucial role in the transmission of transgenerational metabolic risk susceptibility, underscoring the imperative for meticulously devised treatment strategies that are profoundly important for the well-being of future generations.

As previously stated, the complications of GDM in both the short and long term are intricately linked to the intrauterine environment. The placenta, an organ of paramount importance, plays a pivotal role in maintaining the balance of the maternal environment during pregnancy.[13] Operating through the orchestrated release of adipokines, cytokines, hormones, and other regulatory factors, the placenta exerts finely tuned control over insulin sensitivity and glucose uptake, thereby exerting a profound influence on the adverse outcomes of GDM.[14,15] The maintenance of placental health relies on the optimal functioning of mitochondria and dysfunctional mitochondria interlace pathological processes, including disruptions in glucose metabolism and insulin resistance.[16]

Mitochondria are highly dynamic organelles, which undergo continuous cycles of fusion and fission to maintain cellular equilibrium throughout pregnancy.[17,18] Mitochondrial fusion is facilitated by three significant GTP-hydrolyzing enzymes, namely, Mitofusin 1 and 2 (MFN1 and MFN2) modulated outer membrane (OM) fusion and optic atrophy 1 (OPA1) modulated inner membrane fusion.[17,19,20] The pivotal mediator of mitochondrial fission is dynamin-related protein 1 (DRP1), which mobilized from the cytosol to the mitochondrial surface in response to cellular demands, catalyzing GTP hydrolysis and promoting mitochondrial constriction and fission.[21] The adaptors present on the OM that interact with DRP1 include mitochondrial fission factor (MFF), fission 1 (FIS1), and mitochondrial dynamics proteins of 49 kDa (MiD49) and 51 kDa (MiD51).[22–24] (Figure 1) Mitochondrial fission orchestrates mitochondrial biogenesis and mitophagy, while mitochondrial fusion is responsible for preserving the proper distribution of mitochondrial DNA (mtDNA) and metabolic substrates.[25] Under various metabolic or environmental stress conditions, the equilibrium of mitochondrial dynamics tilts toward fission, which can trigger mitochondrial dysfunction marked by mtDNA damage, mutations, and the release of proapoptotic proteins.[26] In the context of the placentae of women with GDM, heightened mitochondrial ROS levels, reduced oxidative phosphorylation rates, and diminished mitochondrial content have been

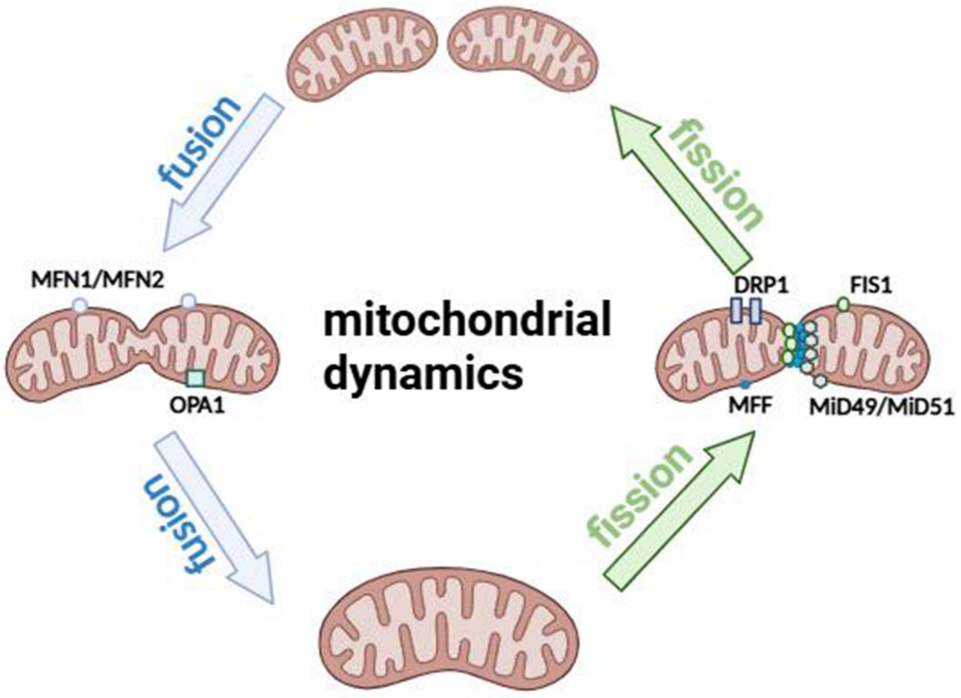

Figure 1 Mitochondrial dynamics. Mitochondrial fusion is facilitated by OPA1, MFN1 and MFN2. The pivotal mediators of mitochondrial fission are DRP1, MFF, FIS1, MiD49 and MiD51.

discerned, which means that mitochondrial dysfunction exists in the placentae of women with GDM.[27] In conclusion, mitochondrial dysfunction potentially contributes to the establishment of a hyperglycemic milieu in GDM, but whether mitochondrial fission, an important process underlying mitochondrial dysfunction, is intricately associated with the emergence of a hyperglycemic intrauterine environment remains an unresolved question.

Previous studies have demonstrated that the incidence of diabetes complications is associated with increased mitochondrial fission.[24,28] Mitochondria in diabetic kidneys tend to undergo increased fission, the fusion process is inhibited;[29] the pathological processes of diabetic kidneys can be effectively reversed by inhibiting mitochondrial fission.[30,31] The pivotal role of cardiac mitochondria in diabetic cardiomyopathy has been well-established, highlighting their potential as therapeutic targets for cardiovascular disease.[32] Mitochondrial dynamics are a new target for addressing diabetes mellitus and its associated complications, but few studies have investigated the interplay between mitochondrial dynamics and GDM. Therefore, further exploration of the involvement of mitochondrial dynamics in the physiological and pathological processes of GDM is warranted.

Current medication used for GDM includes insulin, metformin and sulfonylureas,[33] which mainly work on the beta cell function of pancreas. However, metformin and sulfonylureas can cross the placenta and a few data showed that long term safety for offspring exists some concern.[34] Evidence indicates that using metformin during the pregnancy susceptibility to obesity and metabolic syndrome may be alter in childhood.[35] Since the placenta is an important organ for maternal-fetal communication, focus on the placenta may get the new therapy that is good for the offspring in later life. Currently, such drugs are lacking.

The primary objective of this investigation was to investigate alterations in mitochondrial dynamics within GDM placenta, and HTR8/SVneo cell line was used to explore the implications of mitochondrial dynamics for the high-glucose intrauterine environment and insulin resistance. In doing so, this study has the potential to unveil a novel avenue for improving the hyperglycemic intrauterine milieu, consequently mitigating the unfavorable impacts on offspring.

Methods and Materials

Mitochondrial Dynamic-Related Proteins in GDM Placenta

Sample Inclusion and Exclusion Criteria

In total, 40 placental tissues were obtained from pregnant women undergoing cesarean section at term who were diagnosed with GDM (n = 20) and those with normal pregnancies (NP, n = 20). All participants with other pregnancy complications or concurrent conditions, including prediabetes, hypertension, thyroid disease, and other metabolic diseases, were excluded. Patient treated with insulin or metformin were also excluded to eliminate the effects of drugs on the results. The diagnosis of GDM was confirmed through a 75 g oral glucose tolerance test (OGTT) administered between the 24th and 28th weeks of gestation, adhering to the diagnostic criteria stipulated by the International Association of Diabetes and Pregnancy Study Groups (IADPSG).[36] All individuals who participated in the study informed consent prior to their inclusion. The ethical considerations governing this research were evaluated and approved by the Ethics Committee of Tongji Hospital, Tongji Medical College of Huazhong University of Science and Technology Ethics Committee (Ethics batch number: TJ-IRB20220104).

Sample Collection and Processing

The samples measuring approximately 1cm×1cm×1cm were cut from the placenta, and the sample are stored at −80°C. For analysis, the samples are removed from −80°C freezer and lysed using RIPA buffer supplemented with protease and phosphatase inhibitors. Protein quantification was performed using a BCA protein assay kit (P0012, Beyotime, China). Cut another pieces of placenta samples and fixed in 4% polyoxymethylene.

Western Blotting

Each protein sample (20 μg) was subjected to electrophoresis on an 11% SDS–PAGE gel and subsequently transferred onto PVDF membranes. Subsequently, the membranes were blocked with 5% nonfat milk and then incubated overnight at 4 °C with primary antibodies specific for β-tubulin (1:5000, 66,240-1-Ig, Proteintech, China) and mitochondrial dynamic-related protein includes OPA1 (1:1000, ab157457, Abcam, UK), MFN1 (1:1000, 13,798-1-AP, Proteintech,

China), MFN2 (1:1000, ab124773, Abcam, UK), MFF (1:2000, 17,090-1-AP, Proteintech, China), FIS1 (1:1000, 10,956-1-AP, Proteintech, China) and DRP1 (1:1000, 12,957-1-AP, Proteintech, China). The following day, incubated the membranes with the corresponding secondary antibodies at ambient temperature for 1 h. A chemiluminescence solution (K-12045-D50, Advansta, USA) was prepared, and each protein band was visualized using a G:BOX Chemi XRQ (Syngene, UK). The intensities of the protein bands were quantified and normalized to that of the β-tubulin band by ImageJ software.

Immunofluorescence

Placental tissues fixed in 4% polyoxymethylene were dehydrated for paraffin embedding. Placental samples were subjected to immunostaining with an antibody targeting MFF (1:200), followed by visualization using an immunofluorescence microscope (Olympus BX53, Japan). The mean fluorescence intensity of MFF expression within the placental samples was subsequently quantified using ImageJ.

Cell Experiment

The Impact of Hyperglycemic on Mitochondrial Dynamic-Related Protein of HTR8/SVneo

Cell Culture and Treatment

HTR8/SVneo cells were procured from the China Center for Type Culture Collection (CCTCC) and maintained in Roswell Park Memorial Institute (RPMI) 1640 medium (Invitrogen, USA) supplemented with 10% fetal bovine serum (CellMax, China) at 37 °C in an incubator with a 5% CO2 atmosphere. Following incubation with normal (NG, 11.1 mm glucose) or high glucose (HG, 25 mm glucose) for 48 h, the cells were harvested for subsequent analysis.

Western Blotting

Lysed HTR8/SVneo cells by using RIPA buffer supplemented with protease and phosphatase inhibitors. Each protein sample was subjected to electrophoresis, block with 5% nonfat milk and then incubated overnight with primary antibodies β-tubulin, OPA1, MFN1, MFN2, MFF, FIS1 and DRP1. The following day, incubated the membranes with the corresponding secondary antibodies, and each protein band was visualized using chemiluminescence solution and G:BOX Chemi XRQ.

The Impact of Overexpress or Knockdown MFF on Mitochondria

Cell Culture and Treatment

Knockdown of MFF was achieved in HTR8/SVneo cells through transfection with short hairpin RNA (shRNA)–MFF following the manufacturer's instructions. Three small hairpin RNAs (shRNA-MFF 1, shRNA-MFF 2, and shRNA-MFF 3) were used to suppress MFF expression in HTR8/SVneo cells. Using Western blotting to evaluate the gene expression silencing efficiency, and the results revealed that shRNA-MFF 2 was the most effective. Therefore, in subsequent experiments, HTR8/SVneo cells were transfected with sh-MFF 2 to inhibit the expression of MFF.

The plasmid pCMV-MFF and a negative control vector were procured from Biosune Biotechnology (Shanghai Co., Ltd., China) and utilized for the transfection of HTR8/SVneo cells to overexpress MFF expression.

Immunofluorescence

HTR8/SVneo cells cultured in confocal dishes were immunostained with TOM20 (a mitochondrial marker, 1:500), and the resultant images were captured utilizing a confocal microscope (Olympus FV1000, Japan). The mitochondrial lengths of HTR8/SVneo cells were subsequently quantified using ImageJ.

The Impact of Overexpress or Knockdown MFF on Insulin Signaling Pathway

Cell Culture and Treatment

Incube HTR8/SVneo with normal (NG, 11.1 mm glucose) or high glucose (HG, 25 mm glucose) for 48 h, additionally, to activate the signaling pathways downstream of insulin, HTR8/SVneo cells were treated with insulin (100 nmol/L). After the incubation period, the cells were harvested for subsequent analysis.

Western Blotting

The PI3K-AKT signaling pathway plays an important role in insulin metabolism. PI3K is a heterodimer composed of 110 kDa catalytic subunit and 85 kDa regulatory subunit. P110 has different isomers, including P110α and P110β, which regulate cell growth, metabolism, and insulin resistance. The P85 subunit acts as an adapter to pair the catalytic p110 subunit to the activated receptor, and studies have shown that overexpression of P85 can lead to insulin resistance. Therefore, using Western blot to evaluate the expression of p110α(1:500, #4249, Cell Signaling Technology, USA), p110β(1:1000, #3011, Cell Signaling Technology, USA), p-p85(1:1000, #17366, Cell Signaling Technology, USA), p85 (1:500, #4257, Cell Signaling Technology, USA), p-AKT^{Ser473} (1:2000, #4060, Cell Signaling Technology, USA) and AKT(1:2000, #9272S, Cell Signaling Technology, USA) and determine the impact of MFF on insulin signaling pathway.

The Impact of Overexpress and Knockdown MFF on Glucose Uptake

Western Blotting

Since insulin-induced glucose transport in the placenta is facilitated by Glucose transporter 4(GLUT4), Western blot is used to evaluate the expression of GLUT4 (1:1000, 66,846-1-Ig, Proteintech, China) and determine glucose transport in HTR8/SVneo.

Glucose Consumption

The glucose levels within the culture medium of HTR8/SVneo cells were quantified using a glucose assay kit (Sigma–Aldrich, USA) according to the manufacturer's instructions. Glucose consumption was calculated using a standard calibration curve and normalized to the corresponding protein concentrations.

Statistical Analysis

The continuous variables are expressed as the means ± standard deviations (SDs). SPSS 25.0 was used for statistical analyses, and graphical representations were created using GraphPad Prism 8.0. Comparisons between two independent groups were performed using Student's *t* test or a nonparametric test. Two-way ANOVA was utilized to compare multiple groups and treatments, while one-way ANOVA was used to compare multiple groups. A P value less than 0.05 was considered to indicate statistical significance.

Results

Clinical and Laboratory Parameters of Pregnant Women in the GDM and NP Groups

A total of 40 pregnant women were included as participants in the study. The mean age within the normal pregnancy (NP) group, comprising 20 pregnant women, was determined to be 32.65 ± 2.08 years. In contrast, the mean age of the GDM group, which also included 20 pregnant women, was 34.40 ± 3.93 years. Although there was no significant difference in maternal body mass index (BMI) between the GDM and NP groups (P=0.955), pregnant women with GDM exhibited notably elevated plasma glucose levels in comparison to their NP counterparts (Table 1).

Table 1 Clinical and Laboratory Data of Pregnant Women

	NP (n=20)	GDM (n=20)	*P* value
Maternal age (years)	32.65±2.08	34.40±3.93	0.086
Gestational age (weeks)	38.05±1.06	37.47±1.90	0.239
Prepregnancy BMI (kg/m^2)	21.80±2.84	21.86±2.64	0.955
FPG (mmol/L)	4.575±0.34	5.063±0.71	0.03*
1 h OGTT (mmol/L)	7.183±1.34	9.852±1.16	< 0.001***
2 h OGTT (mmol/L)	6.868±0.93	8.751±1.72	0.002**

Note: *P<0.05, **P<0.01, ***P<0.001.

Abbreviations: GDM, gestational diabetes mellitus; NP, normal pregnancy; BMI, body mass index; FPG, fasting plasma glucose; OGTT, oral glucose tolerance test.

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Expression of the MFF Protein in the Placental Tissues of Women in the GDM and NP Groups

To determine alterations in the expression levels of mitochondrial dynamics-related proteins in placental tissues, we conducted Western blotting analyses to determine the expression levels of these proteins in placental tissues. MFF expression was significantly elevated in the placental tissues of the GDM group (Figure 2A-D). Moreover, immunofluorescence was employed to visualize the localization of MFF expression (depicted in red) in the placenta, and the results indicated that MFF expression was predominantly localized to trophoblasts. Intriguingly, our observations also revealed a conspicuous increase in MFF expression within the placental tissues of the GDM group compared to those of the NP group (Figure 2E and F). The expression of the other mitochondrial dynamic-related proteins did not significantly differ from those in the NP group.

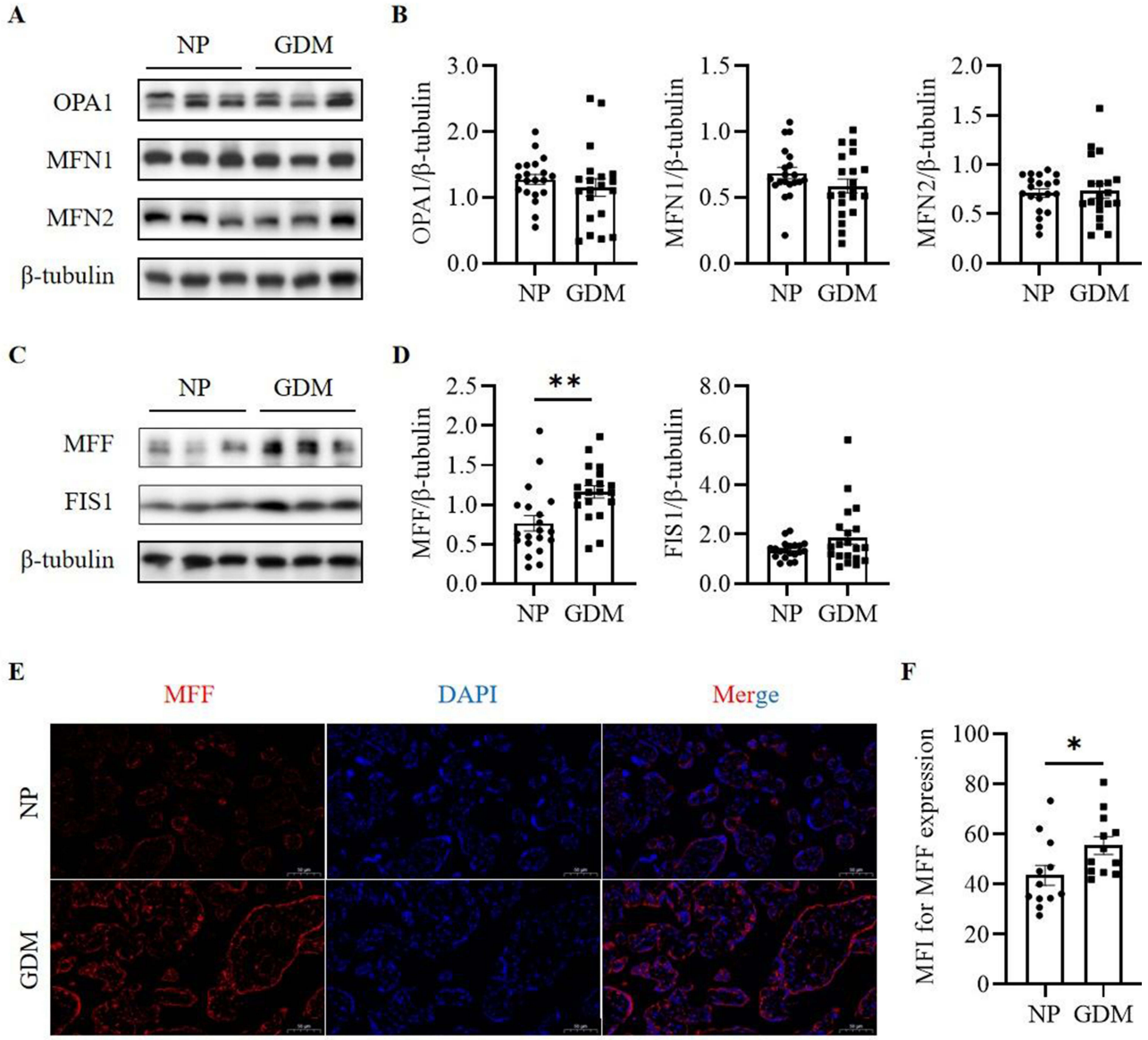

Figure 2 Expression of MFF in the placental tissues of pregnant women in the GDM and NP groups. (**A-D**) Representative images and quantitative analyses of mitochondrial dynamics-related protein expression determined by Western blotting in placental tissues of the GDM (n=20) and NP (n=20) groups. (**E** and **F**) Representative images and quantitative analyses of immunofluorescence staining of MFF expression (red) in placental tissues from the GDM (n=20) and NP (n=20) groups. * P<0.05, **P<0.01. The data are expressed as the means ± SEMs.

Expression of Mitochondrial Dynamics–Related Proteins in a High-Glucose Environment

Next, we investigated the expression of proteins related to mitochondrial dynamics in HTR8/SVneo cells treated with high glucose. Interestingly, the analysis revealed a noteworthy reduction in the expression of the mitochondrial fusion-related proteins OPA1, MFN1, and MFN2 in the high glucose-treated groups compared to the control groups (Figure 3A and B). In addition, the protein expression levels of both DRP1 and MFF increased in HG-treated cells compared to NG-treated cells (Figure 3C and D).

Knockdown and Overexpression of MFF Affects the Lengths of Mitochondria

In this study, we then investigated the role of MFF in governing mitochondrial fission in HTR8/SVneo cells, highlighting its importance in this process. We used three small hairpin RNAs (shRNA-MFF 1, shRNA-MFF 2, and shRNA-MFF 3) to suppress MFF expression in HTR8/SVneo cells. Furthermore, Western blotting was used to evaluate the gene expression silencing efficiency, and the results revealed that shRNA-MFF 2 was the most effective at reducing the MFF protein expression level (Figure 4A and C). Therefore, in subsequent experiments, HTR8/SVneo cells were transfected with sh-MFF 2 to inhibit the expression of MFF. The data presented in Figure 4E and F demonstrate that the attenuation of MFF expression resulted in a reduction in mitochondrial fission, as evidenced by the elongation of mitochondria in the sh-MFF group compared to those in the shNC group. In addition, the MFF protein was overexpressed in HTR8/SVneo cells using pCMV (Figure 4B and D). The increase in MFF expression had a demonstrable effect, significantly increasing mitochondrial fission within HTR8/SVneo cells (Figure 4G and H). These data collectively underscore the regulatory influence exerted by MFF on mitochondrial fission in HTR8/SVneo cells.

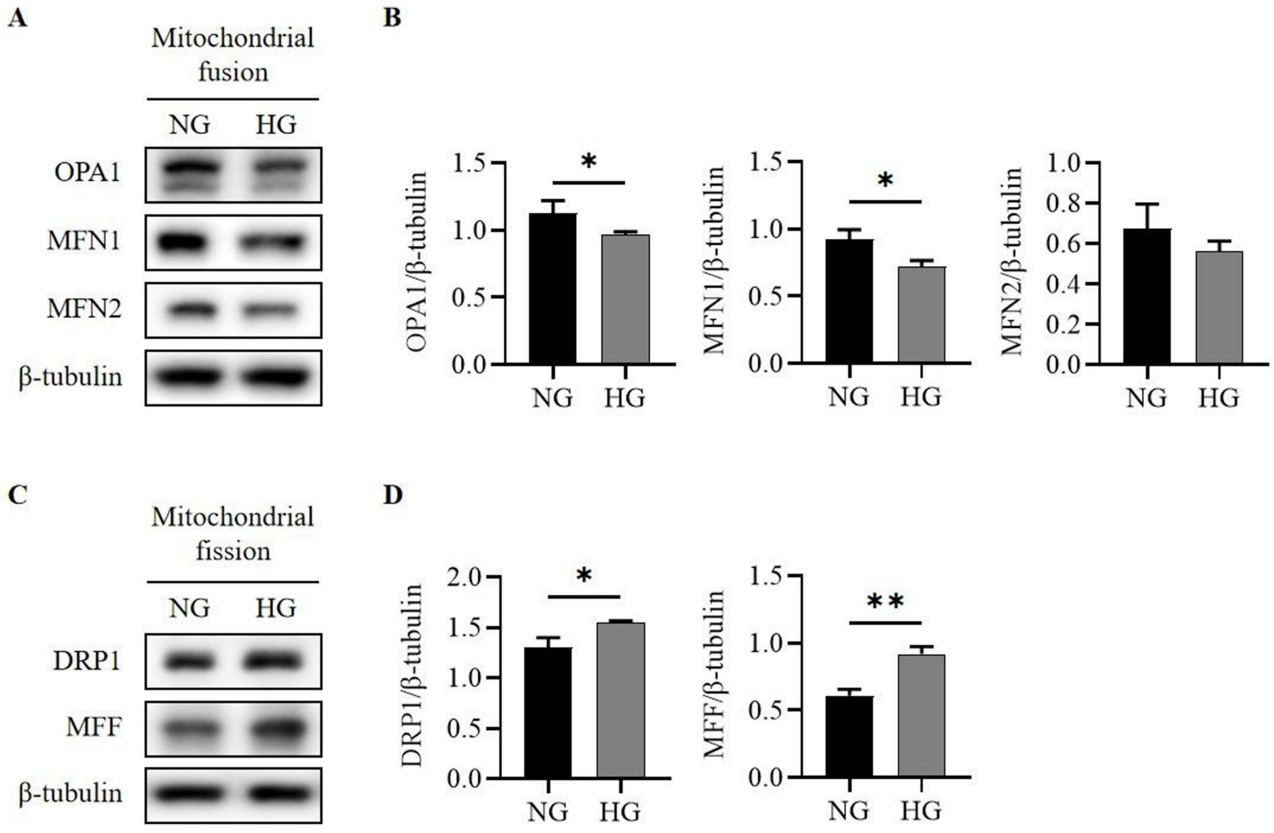

Figure 3 Representative images and quantitative analyses of mitochondrial fusion-related protein expression in high glucose-treated HTR8/SVneo cells determined by Western blotting (**A** and **B**). Representative images and quantitative analyses of mitochondrial fission-related protein expression (**C** and **D**). *P<0.05, **P<0.01. The data are expressed as the means ± SEMs.

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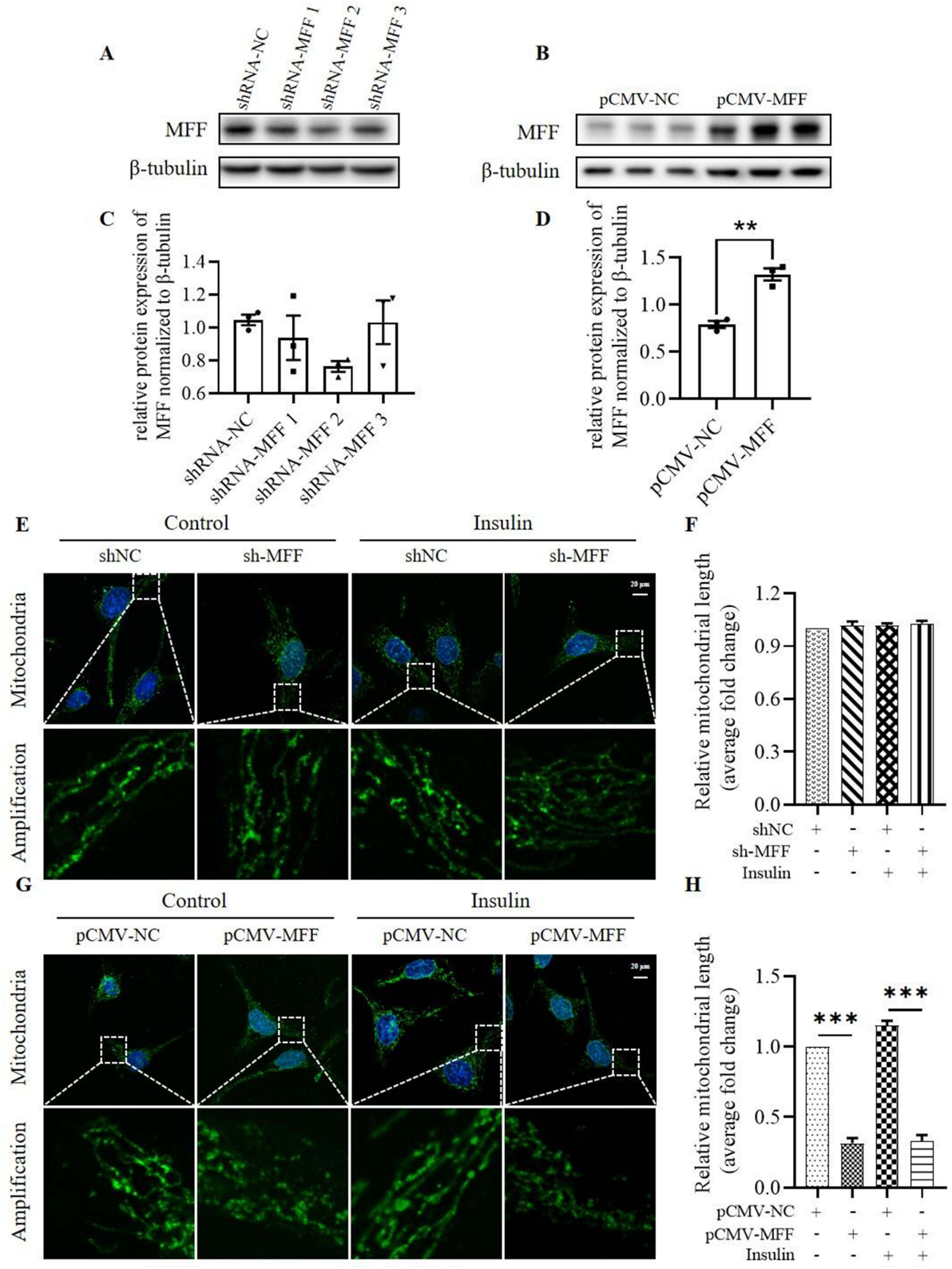

Figure 4 Representative images and quantitative analyses of MFF protein expression. (**A-D**) Knockdown (sh-MFF) and overexpression (pCMV-MFF) of MFF affect the lengths of mitochondria. (**E** and **G**) Representative images showing mitochondrial morphology. (**F** and **H**) Relative mitochondrial length (average fold change). **P<0.01, ***P<0.001.

Knockdown and Overexpression of MFF Affects the Insulin Signaling Pathway in Trophoblasts

To elucidate the impact of MFF-mediated mitochondrial fission on insulin sensitivity, Western blotting analysis was employed to determine the expression levels of key proteins within the insulin signaling pathway. Notably, upon MFF knockdown, there was a significant increase in the phosphorylation of p-AKT^{Ser473} in the sh-MFF+insulin group, which was markedly greater than that in the sh-NC+insulin group (Figure 5A and B). Moreover, overexpression of MFF inhibited the phosphorylation of p-AKT^{Ser473} compared to that in the pCMV-MFF+insulin group (Figure 5C and D). In

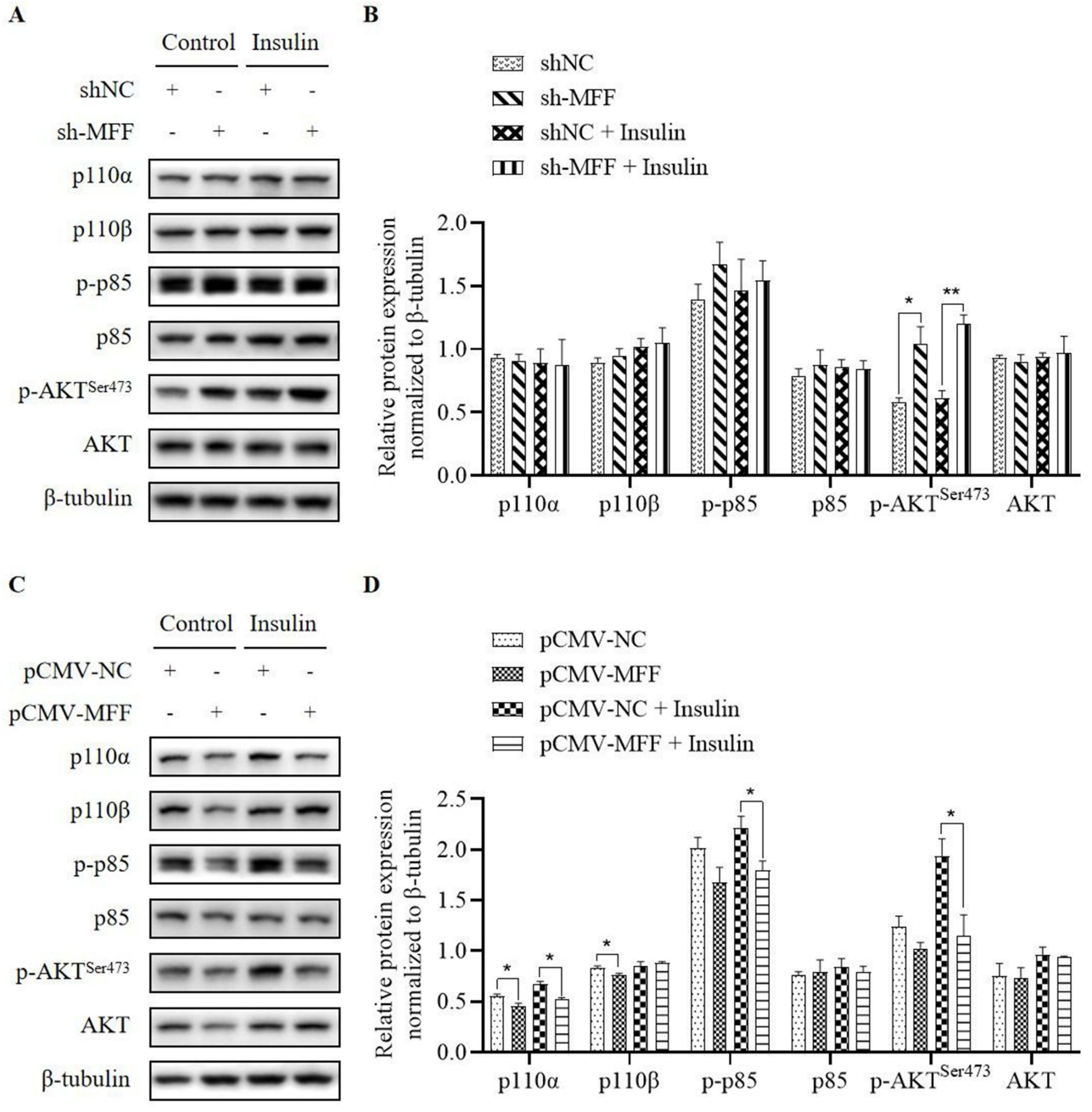

Figure 5 Expression of key proteins in the insulin signaling pathway when MFF expression is knocked down (sh-MFF) or overexpressed (pCMV-MFF). Representative images and quantitative analyses of key protein expression were determined by Western blotting in HTR8/SVneo cells. (**A-D**) *P<0.05 **P<0.01. Data are expressed as the means ± SEMs.

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addition, heightened expression of MFF was associated with a decrease in the expression and phosphorylation levels of p110α, p110β and p-p85, respectively.

Knockdown and Overexpression of MFF Affects Glucose Uptake in Trophoblasts

To determine whether heightened mitochondrial fission is intricately linked to the regulation of glucose uptake and transport, we meticulously assessed the changes in glucose consumption in HTR8/SVneo cells subjected to MFF expression silencing or overexpression. Additionally, the expression of GLUT4 was investigated in this context. Notably, within the sh-MFF+ insulin group, a significant increase in glucose uptake was evident in comparison to that in the si-NC+insulin group (Figure 6A). In addition, the expression levels of GLUT4 were markedly elevated in the sh-MFF+insulin group compared with those of the sh-NC+insulin group (Figure 6B and C). Conversely, upon MFF overexpression, the results showed the opposite trend (Figure 6D-F).

Discussion

Gestational diabetes mellitus (GDM) is a metabolic disorder that impacts the immediate and future well-being of both mothers and their offspring as a result of maternal insulin resistance and exposure to high levels of glucose in the intrauterine environment.[6–8,12] Numerous studies have indicated the significance of mitochondrial homeostasis in the regulation of insulin sensitivity and glycometabolism.[16] In this study, we found an aberrance of mitochondrial homeostasis in trophoblast cells of the placentae of women with GDM, characterized by reduced expression of key proteins involved in mitochondrial fusion and elevated expression of key proteins associated with mitochondrial fission. Among these, the most notably significant difference was observed in the expression of MFF, a crucial regulator of mitochondrial fission.

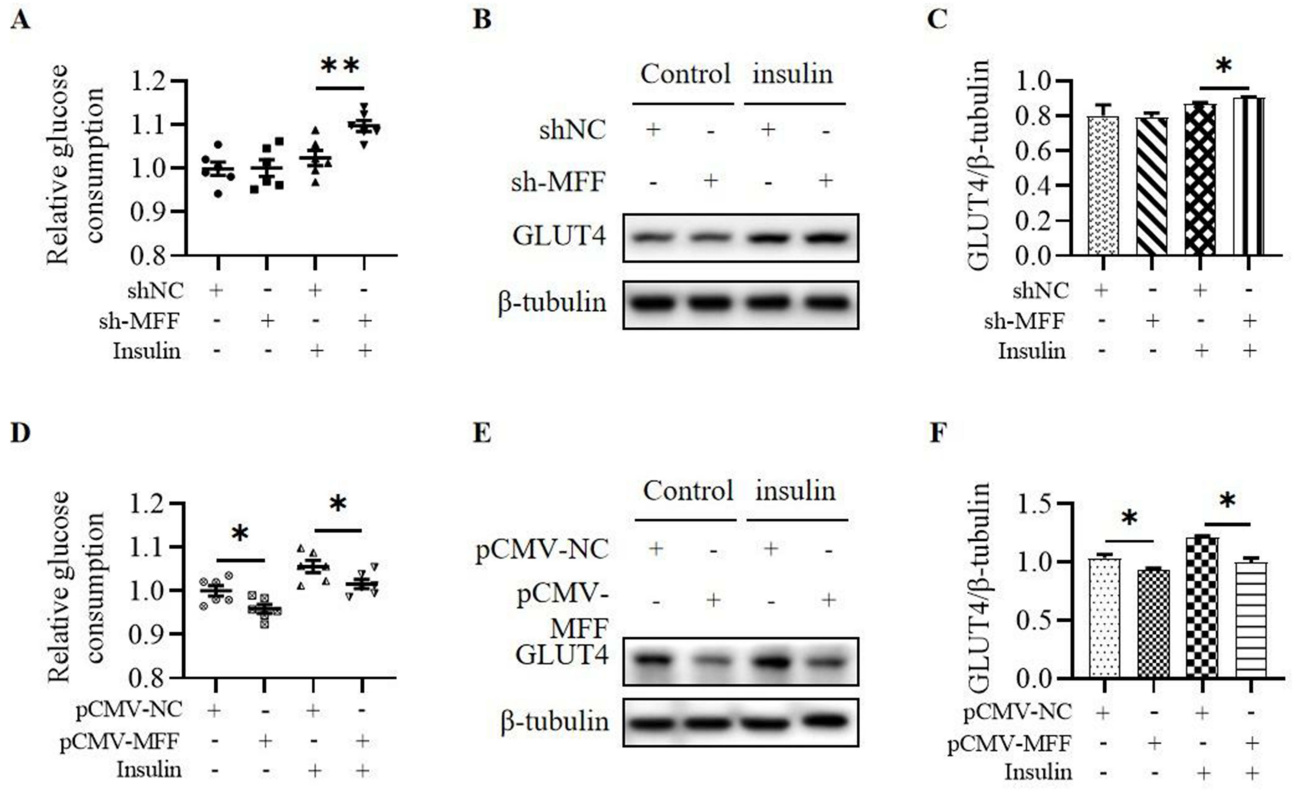

Figure 6 Glucose uptake and GLUT4 expression in HTR8/SVneo cells with MFF expression knockdown (sh-MFF) or overexpression (pCMV-MFF). Relative quantitative analyses of glucose consumption. (**A** and **D**) Representative images and quantitative analyses of key protein expression were determined by Western blotting in HTR8/SVneo cells. (**B, C, E, F**) *P<0.05. The data are expressed as the means ± SEMs.

MFF is an important regulator of mitochondrial fission, as evidenced by its deletion resulting in decreased recruitment of DRP1 to mitochondria and hindered fission, while its overexpression leads to heightened mitochondrial fragmentation.[17] Given the significance of mitochondria in glucose metabolism, elevated blood glucose levels can result in an increase in mitochondrial load, prompting excessive mitochondrial fission and fragmentation across various cells. This aberrant process can culminate in heightened reactive oxygen species (ROS) production and subsequent mitochondrial dysfunction.[37,38] Consistent with our findings, previous studies have indicated that MFF participate in the pathogenesis and progression of abnormal glucose metabolism-related diseases. Previous research has demonstrated that in diabetic nephropathy, hyperglycemia stimulates MFF-mediated mitochondrial fission via upregulation of NR4A1 expression, resulting in an imbalance in mitochondrial dynamics, glomerular apoptosis, and impaired renal function.[39] However, the impact of RNA interference or MFF expression knockout on human renal mesangial cell function has not been directly investigated. A study on obesity highlighted the significant contribution of hepatic MFF-dependent mitochondrial fragmentation to the development of glucose metabolism disturbances induced by a high-fat diet (HFD).[40] However, the study did not directly measure the alterations of MFF expression levels in HFD-fed mice compared to those in mice fed a normal diet. Our data revealed that HG exposure triggers elevated MFF-induced mitochondrial fission in trophoblast cells and subsequently leads to insulin resistance.

GDM is characterized by insulin resistance across various tissues and organs, and insulin resistance is associated with impaired insulin signaling pathways. The PI3K/AKT signaling pathway is integral to the regulation of insulin metabolism, including the control of glucose uptake, cellular metabolism, and glycogen synthesis. Mitochondrial dynamics have been proposed to be a bridge linking mitochondrial dysfunction to insulin resistance.[41] The causal role of mitochondrial dynamics in insulin resistance is likely mediated through mtROS[42] or lipid oversupply.[43] Within the realm of mitochondrial dynamics-related proteins, ablation of MFF expression alone has been shown to safeguard against mitochondrial fragmentation in the hepatic tissues of mice and to improve systemic insulin sensitivity.[40] Furthermore, our study revealed that during the activation of MFF-mediated mitochondrial fission in HTR8/SVneo cells, the expression of p-AKT^{Ser473}, p110α, p110β and p-p85 was significantly inhibited, leading to impairment of the insulin signaling pathway. Given that impaired insulin signaling pathways commonly lead to insulin resistance, we conclude that activated mitochondrial fission contributes to the progression of insulin resistance in the placental trophoblast cells of women with GDM.

During pregnancy, fetal glucose is exclusively derived from the mother. Elevated glucose levels in the uterine environment can lead to an overabundance of fetal energy substrate, and the chances of obesity, heart enlargement and macrosomia in the fetuses of women with GDM also increase.[44] Glucose transport in the placenta is facilitated by Glucose transporters (GLUTs), whereas insulin-induced glucose transport is predominantly attributed to the translocation of GLUT4.[45] Mitochondrial inhibition has been shown to promote the translocation of GLUT4 to the cell membrane and reverse insulin resistance.[42] Consequently, silencing MFF expression can enhance GLUT4 expression and cellular glucose uptake, potentially ameliorating the hyperglycemic intrauterine environment and offering a novel therapeutic approach for improving the adverse outcomes of the progeny of women with GDM.

All in all, placental MFF-mediated mitochondrial fission plays a role in glucose consumption by the insulin signalling pathway. Since the placenta is an important organ for material exchange between the fetus and the mother, MFF-mediated mitochondrial fission contributes to the development of the fetus. Clarifying the effects of drugs on the placenta may help to evaluate their role in fetal development. For example, metformin, a commonly used drug for GDM treatment, has an effect on mitochondrial dynamic. Metformin can phosphorylate AMPK/DRP1 to induce mitochondrial fission,[46] downregulate p32 and OPA1 to induce mitochondrial fission in non-small cell lung cancer,[47] while inhibit mitochondrial fission in insulin-resistant (IR) cells.[48] However, little attention has been paid to the effect of metformin on placental trophoblast cells. At present, the long-term effects of metformin on the fetus have attracted much attention. Clarifying the effect of metformin on placental mitochondrial fission may help to answer this question.

However, our study still has certain limitations that need to be addressed. First, although we observed that MFF-mediated mitochondrial fission affects AKT expression, elucidation of the specific pathways regulating insulin activity is necessary. Second, the impact of mitochondrial fission on insulin resistance in vivo remains ambiguous, and there is a dearth of animal-based investigations on this topic.

Conclusion

In summary, our study demonstrates that MFF-mediated mitochondrial fission contributes to the progression of GDM through a reduction in insulin sensitivity and an increase in glucose consumption, which implies that targeting MFF may be a novel therapeutic approach for GDM. Nevertheless, further investigation is warranted to validate the association between MFF-mediated mitochondrial fission and GDM.

Ethics Statement

Approval of the research protocol: The research protocol was approved by the Medical Ethics Committee of the Tongji Hospital of Huazhong University of Science and Technology (Ethics batch number: TJ-IRB20220104).

Clinical Samples

Our study complies with the declaration of Helsinki.

Acknowledgments

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Informed Consent

All participants signed their written informed consent.

Disclosure

The authors declare no conflict of interest.

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