

Silencing of *DsbA-L* gene impairs the PPAR γ agonist function of improving insulin resistance in a high-glucose cell model*

Xuan ZHOU, Jia-qi LI, Li-jie WEI, Meng-zhou HE, Jing JIA,

Jing-yi ZHANG, Shao-shuai WANG^{†‡}, Ling FENG^{†‡}

Department of Obstetrics and Gynecology, Tongji Hospital, Tongji Medical College,
 Huazhong University of Science and Technology, Wuhan 430030, China

[†]E-mail: fltj007@163.com; colombo2008@sina.com

Received Aug. 4, 2020; Revision accepted Oct. 18, 2020; Crosschecked Nov. 26, 2020

Abstract: Disulfide-bond A oxidoreductase-like protein (DsbA-L) is a molecular chaperone involved in the multimerization of adiponectin. Recent studies have found that DsbA-L is related to metabolic diseases including gestational diabetes mellitus (GDM), and can be regulated by peroxisome proliferator-activated receptor γ (PPAR γ) agonists; the specific mechanism, however, is uncertain. Furthermore, the relationship between DsbA-L and the novel adipokine chemerin is also unclear. This article aims to investigate the role of DsbA-L in the improvement of insulin resistance by PPAR γ agonists in trophoblast cells cultured by the high-glucose simulation of GDM placenta. Immunohistochemistry and western blot were used to detect differences between GDM patients and normal pregnant women in DsbA-L expression in the adipose tissue. The western blot technique was performed to verify the relationship between PPAR γ agonists and DsbA-L, and to explore changes in key molecules of the insulin signaling pathway, as well as the effect of chemerin on DsbA-L. Results showed that DsbA-L was significantly downregulated in the adipose tissue of GDM patients. Both PPAR γ agonists and chemerin could upregulate the level of DsbA-L. Silencing *DsbA-L* affected the function of rosiglitazone to promote the phosphatidylinositol 3-kinase (PI3K)-protein kinase B (PKB)/AKT pathway. Therefore, it is plausible to speculate that DsbA-L is essential in the environment of PPAR γ agonists for raising insulin sensitivity. Overall, we further clarified the mechanism by which PPAR γ agonists improve insulin resistance.

Key words: Disulfide-bond A oxidoreductase-like protein (DsbA-L); Peroxisome proliferator-activated receptor γ (PPAR γ); Chemerin; Insulin signaling pathway; Gestational diabetes mellitus

<https://doi.org/10.1631/jzus.B2000432>

CLC number: R714.256

1 Introduction

Disulfide-bond A oxidoreductase (DsbA) is a protein disulfide isomerase that contributes to the formation of disulfide bonds in *Escherichia coli* (Wang and Scherer, 2008). Glutathione S-transferase

kappa (GST- κ) shares high structural similarities to DsbA, and hence it is also called “DsbA-like protein (DsbA-L).” The *DsbA-L* gene, located in the mitochondria, is essential to maintain mitochondrial homeostasis. The downregulation of DsbA-L could trigger the imbalanced secretion of mitochondrial DNA (mtDNA), thereby inducing mitochondrial dysfunction (Deng et al., 2020). Furthermore, it has been reported that the lack of DsbA-L could activate the cyclic guanosine-adenosine monophosphate synthase-stimulator of interferon genes (cGAS-STING) pathway to enhance inflammation and insulin resistance (Bai et al., 2017). It was supposed that polymorphisms

[‡] Corresponding authors

* Project supported by the National Key Research and Development Program of China (Nos. 2016YFC1000405 and 2018YFC1002903)

 ORCID: Ling FENG, <https://orcid.org/0000-0001-8833-5574>; Shao-shuai WANG, <https://orcid.org/0000-0003-0315-7866>

© Zhejiang University and Springer-Verlag GmbH Germany, part of Springer Nature 2020

of the *DsbA-L* gene might be associated with metabolic diseases (Gao et al., 2009).

Gestational diabetes mellitus (GDM) is defined as a type of metabolic disease, characterized by hyperglycemia at the onset of pregnancy. The worldwide prevalence of GDM has reached approximately 15% to date (Griffith et al., 2020), with the risk of type 2 diabetes mellitus (T2DM) getting higher with the extension of follow-up time in GDM patients (Li et al., 2020). Studies have found that adiponectin expression showed constantly lower levels in GDM patients (Lorenzo-Almorós et al., 2019), which is likely related to decreased levels of chaperone DsbA-L. Insulin resistance is the major feature of GDM. The exact roles DsbA-L plays in the mechanism of insulin signaling transduction in GDM are not yet fully understood. Therefore, we established a cell model to mimic GDM placenta by adding glucose at high concentration (25 mmol/L) to the growth medium of a type of human trophoblastic cell lines, namely HTR-8/SVneo cells. The relevant methods have been verified by multiple papers in the literature (Basak et al., 2015; Kuricova et al., 2016; Lampropoulou et al., 2016; Wang et al., 2019).

As a molecular chaperone, DsbA-L has the critical role of dominating the assembly and secretion of adiponectin (Zhou et al., 2010). Interactions between DsbA-L and other adipokines, however, remain to be fully explored. An inflammatory chemokine named chemerin was firstly identified by Gorski et al. (2007), as a novel adipokine. A later review elaborated that chemerin affects lipogenesis and angiogenesis via binding to chemokine-like receptor 1 (CMKLR1) (Helfer and Wu, 2018). Beyond that, chemerin also participates in the regulation of glucose metabolism (Helfer and Wu, 2018). Previous studies by authors of the present work have shown that peroxisome proliferator-activated receptor γ (PPAR γ) agonists can affect insulin signaling pathways by regulating chemerin (Zhou et al., 2020). These agonists could enhance the secretion of adiponectin by binding to the peroxisome proliferator response element (PPRE) in the promoter of *DsbA-L* (Jin et al., 2015). Thiazolidinediones (TZDs), such as rosiglitazone (RSG), are types of drug for the treatment of diabetes, decreasing insulin resistance through the activation of PPAR γ (Lebovitz, 2019).

Given that both DsbA-L and insulin signaling pathways could be dominated by the activation of PPAR γ , we investigated the effect of PPAR γ on downstream molecules of the insulin pathway after silencing *DsbA-L* gene to determine whether DsbA-L affects PPAR γ agonist function. In addition, since DsbA-L is associated with adiponectin multimerization, we explored the relationship between DsbA-L and chemerin, a novel adipokine, to better understand the role of DsbA-L in regulating adipokines other than adiponectin.

2 Materials and methods

2.1 Human samples

A total of 16 samples of subcutaneous adipose tissue were collected from pregnant women who underwent regular obstetric examination and suffered cesarean delivery in Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (Wuhan, China). There were six cases of GDM patients and ten normal pregnancies among these cases. A diagnosis of GDM was determined via 75 g Oral Glucose Tolerance Test (OGTT) according to the standards set by International Association of Diabetes and Pregnancy Study Groups (IADPSG). Pregnancies accompanied by basic illnesses, infectious diseases, and other complications of pregnancy besides GDM were excluded. The present study was approved by the Ethics Committee of Tongji Hospital (No. TJ-IRB20170506).

2.2 Immunohistochemical analysis

Subcutaneous adipose tissue samples were rapidly washed in phosphate-buffered saline (PBS) and preserved in liquid nitrogen for frozen sections. For sample analysis, they were subjected to antigen retrieval after blocking with hydrogen peroxide solution, and incubated with anti-DsbA-L rabbit polyclonal antibody (ab92819; 1:100 (volume ratio); Abcam, UK) at 4 °C overnight. The next day, the secondary antibody was added to these sections and they were stained by diaminobenzidine. Images were recorded at 100-fold magnification and evaluated with Image-Pro Plus 6.0 (Media Cybernetics, Denver, USA), with the average optical densities applied for analysis.

2.3 Cell culture and small interfering RNA transfection

Cells of the HTR-8/SVneo line were obtained from Wuhan Servicebio Technology Co., Ltd., China. Dulbecco's modified Eagle's medium (DMEM) with high glucose concentration (about 25 mmol/L) was applied as the basic medium to mimic circulatory glucose levels of GDM. Additionally, the medium was also supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic. Cells were cultured under the conditions of 37 °C and 5% CO₂. Before addition to the cell medium, small interfering RNA (siRNA) of DsbA-L (si-DsbA-L, target sequence CTGTGCC GGTATCAGAATA, Guangzhou RiboBio Co., Ltd., China) and Lipofectamine[®] 3000 transfection reagents were diluted separately by Opti-MEM[™] Reduced Serum Medium, and mixed for 10 min.

2.4 Western blot analysis

The HTR-8/SVneo cells were lysed in radio immunoprecipitation assay (RIPA) buffer and phenylmethanesulfonyl fluoride (PMSF), and the extracted protein was denatured by boiling at high temperature. Samples were then subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes. Thereafter, all membranes were blocked using 5% bovine serum albumin (BSA) for 1 h, and incubated overnight at 4 °C with the following primary antibodies: anti-PI3K (phosphatidylinositol 3-kinase) p110 β rabbit polyclonal antibody (21739-1-AP; 1:500; Proteintech, China), anti-chemerin rabbit polyclonal antibody (10216-1-AP; 1:200; Proteintech, China), anti-DsbA-L rabbit polyclonal antibody (ab92819; 1:2000; Abcam, UK), anti-AKT2 (protein kinase B (PKB) β) rabbit monoclonal antibody (#3063; 1:1000; Cell Signaling Technology, USA), anti-p44/42 MAPK (mitogen-activated protein kinase) (extracellular signal-regulated kinase 1/2 (ERK1/2))

rabbit monoclonal antibody (#4695; 1:1000; Cell Signaling Technology, USA), anti-phospho-p44/42 MAPK (ERK1/2) rabbit monoclonal antibody (#4370; 1:2000; Cell Signaling Technology, USA), anti- β -actin mouse monoclonal antibody (66009-1-Ig; 1:5000; Proteintech, China), and anti-GAPDH (glyceraldehyde-3-phosphate dehydrogenase) mouse monoclonal antibody (60004-1-Ig; 1:10000; Proteintech, China). The next day, the membrane was incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies for 1 h at room temperature, and subjected to electrochemiluminescence assay with detection by G:BOX Chemi XRQ (Syngene, UK). Quantitative analysis was performed using GeneTools software (Syngene, UK).

2.5 Statistical analysis

The SPSS 25.0 software was used for statistical analysis, and GraphPad Prism 5.0 was applied to draw graphics. On the basis of conforming to the normal distribution, a Student's *t*-test was utilized to compare two independent samples with the mean $\pm$ standard deviation (SD) presented for description. The difference was considered statistically significant, when *P*<0.05.

3 Results

3.1 Clinical metabolic parameters of participants

There were no statistically significant differences in maternal age, weight, or body mass index (BMI) of pregnant women between the GDM group and the control group. However, results of OGTT for the GDM group were significantly higher than those for the control group (Table 1).

3.2 Expression of DsbA-L in the subcutaneous adipose tissue

Research has revealed that DsbA-L exists mainly in the mitochondria and endoplasmic reticulum (ER)

Table 1 Clinical metabolic parameters of participants

Group	Age (year)	Weight (kg)	BMI (kg/m ²)	OGTT 0 h (mmol/L)	OGTT 1 h (mmol/L)	OGTT 2 h (mmol/L)
GDM (<i>n</i> =6)	35.17 $\pm$ 3.66	74.00 $\pm$ 7.72	28.18 $\pm$ 3.24	5.11 $\pm$ 0.43	10.00 $\pm$ 2.70	9.35 $\pm$ 0.98
Control (<i>n</i> =10)	32.70 $\pm$ 3.62	66.55 $\pm$ 7.53	26.26 $\pm$ 2.70	4.55 $\pm$ 0.35	7.46 $\pm$ 1.37	6.32 $\pm$ 1.04
<i>P</i> value	0.210	0.087	0.222	0.013*	0.024*	0.000*
<i>t</i> value	-1.314	-1.886	-1.277	-2.833	-2.527	-5.758

GDM: gestational diabetes mellitus; BMI: body mass index; OGTT: oral glucose tolerance test. All data are expressed as mean $\pm$ standard deviation (SD). **P*<0.05

of adipocytes (Liu et al., 2015). Moreover, DsbA-L is expressed at the highest level in adipose tissue in a location where adiponectin is secreted (Wang and Scherer, 2008). In order to determine the level of DsbA-L in the subcutaneous adipose tissue of GDM patients and normal pregnant women, immunohistochemistry and western blot analyses were performed. We found that DsbA-L existed both in the control group and the GDM group (Figs. 1a and 1b) as opposed to the blank group (Fig. 1c) based on staining, while DsbA-L expression was remarkably reduced in the subcutaneous adipose tissue of GDM patients (Fig. 1d). Western blot showed the same results

(Fig. 1e); quantitative analysis indicated that the GDM group showed significantly lower DsbA-L levels compared with the control group (Fig. 1f).

3.3 Effect of chemerin on DsbA-L expression

In order to investigate the effect of chemerin on DsbA-L, different concentrations of recombinant chemerin (#300-66; PeproTech, USA) were additionally supplemented to high glucose-treated HTR-8/SVneo cells, and the effects of chemerin on DsbA-L expression were observed by western blot analysis. Compared with the control group (without extra chemerin), the protein expression of chemerin was

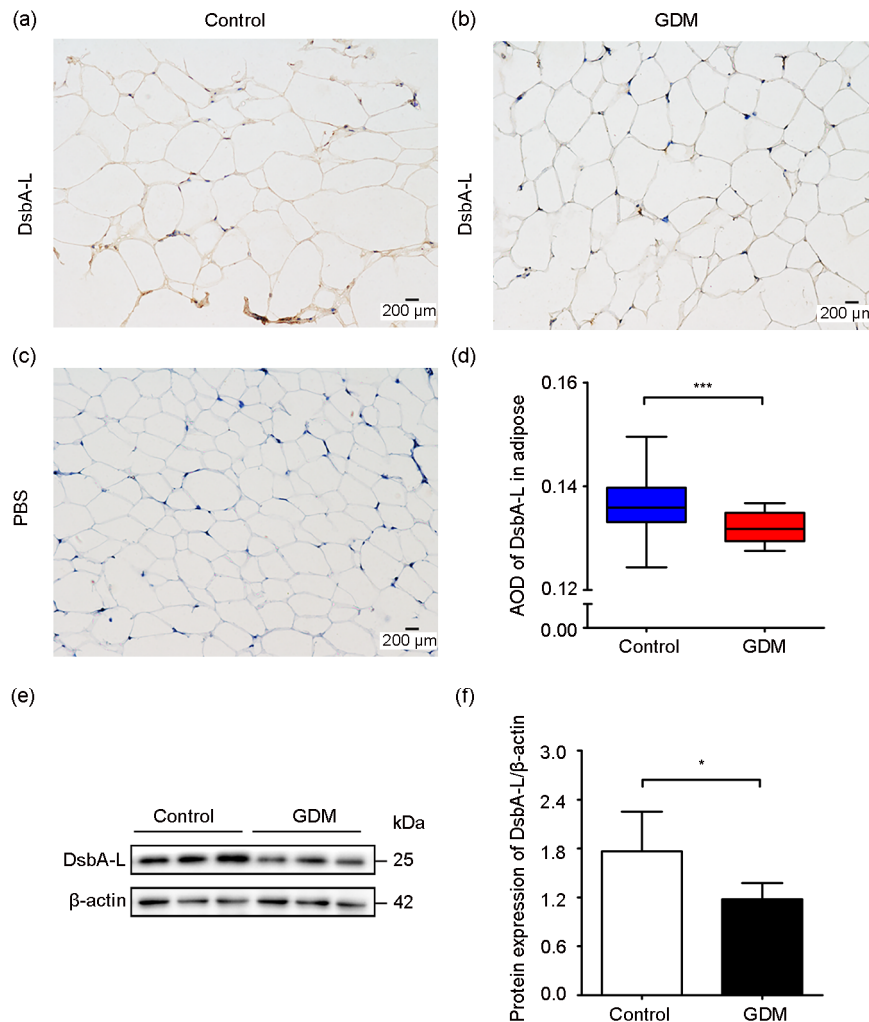


Fig. 1 Expression of DsbA-L in the subcutaneous adipose tissue of GDM patients and normal pregnant women

(a) Level of DsbA-L in normal pregnant women; (b) Expression of DsbA-L in GDM patients; (c) PBS was a substitute for DsbA-L antibody in the blank group; (d) Comparison of DsbA-L levels by immunohistochemistry experiment; (e) Representative image of the immunoblots of adipose tissue samples (25 μ g); (f) Relative quantitative analysis of protein expression of DsbA-L. DsbA-L: disulfide-bond A oxidoreductase-like protein; GDM: gestational diabetes mellitus; PBS: phosphate-buffered saline; AOD: average optical density. Data are shown as mean $\pm$ standard deviation (SD) ($n \geq 3$). * $P < 0.05$, *** $P < 0.001$

gradually elevated with increased dose, and the level of DsbA-L also improved simultaneously with raised chemerin concentration. Chemerin at 80 ng/mL led to the most obvious increase of DsbA-L expression, with a statistically significant difference compared with the control group (Fig. 2).

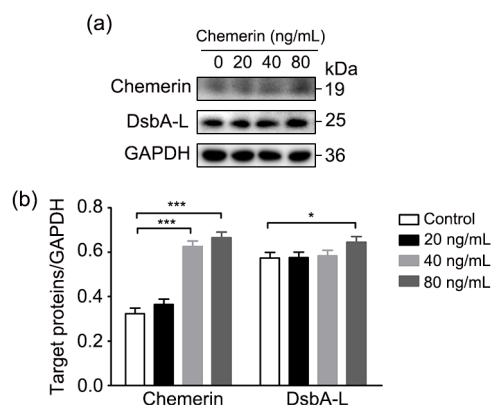


Fig. 2 Expression of DsbA-L upregulated by chemerin

The chemerin concentration gradient consisted of 20, 40, and 80 ng/mL treatments, as well as a control group without additional chemerin. (a) Representative image of the immunoblots of cell samples (50 µg); (b) Relative quantitative analyses of chemerin and DsbA-L. DsbA-L: disulfide-bond A oxidoreductase-like protein; GAPDH: glyceraldehyde-3-phosphate dehydrogenase. Data are shown as mean±standard deviation (SD) ($n=3$). * $P<0.05$, *** $P<0.001$

3.4 Effect of PPAR γ agonists on DsbA-L expression

In order to examine the effect of activation of PPAR γ on DsbA-L, high glucose-treated HTR-8/SVneo cells were subjected to 20 µmol/L RSG (HY-17386; MedChem Express, USA) and 20 µmol/L GW1929 (HY-15655; MedChem Express, USA), two small molecule agonists of PPAR γ , and subsequent western blot analysis following 72 h of incubation. Results showed that protein levels of DsbA-L were significantly increased in the RSG and GW1929 groups (Fig. 3) compared with the control group, suggesting that PPAR γ agonists had an effect of upregulating DsbA-L expression.

3.5 Effect of silencing the *DsbA-L* gene on PPAR γ agonist action of improving insulin resistance

The following treatment was conducted to identify the role of DsbA-L in the process of PPAR γ agonist enhancing insulin signaling pathways. Firstly, HTR-8/SVneo cells were incubated with insulin

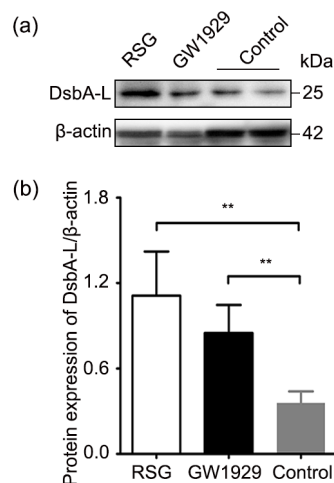


Fig. 3 Protein expression of DsbA-L enhanced by PPAR γ agonists, RSG, and GW1929

(a) Representative image of the immunoblots of cell samples (50 µg); (b) Relative quantitative analysis of DsbA-L. PPAR γ : peroxisome proliferator-activated receptor γ ; DsbA-L: disulfide-bond A oxidoreductase-like protein; RSG: rosiglitazone. Data are shown as mean±standard deviation (SD) ($n=3$). ** $P<0.01$

(100 nmol/L) for 48 h to induce the signaling pathways downstream of insulin. Then, we transfected siRNA into these cells targeting the *DsbA-L* gene (si-DsbA-L group). Six hours later, RSG (20 µmol/L) was added to the cell medium on the basis of silencing the *DsbA-L* gene for the group named si-DsbA-L+RSG. Finally, we carried out western blot analysis after 72 h; the protein signals of PI3K p110 β , AKT2, ERK1/2, and DsbA-L are shown in Fig. 4a. There were no significant changes in the protein expression of PI3K p110 β or total AKT2 (Figs. 4b and 4c). The phosphorylation of ERK1/2 was significantly increased after *DsbA-L* knockdown compared with the negative control (NC, cells transfected with scramble siRNA) group (Fig. 4d). Moreover, the phosphorylation of ERK1/2 was diminished in the si-DsbA-L+RSG group compared with the si-DsbA-L group. In addition, the protein level of DsbA-L was reduced in the si-DsbA-L group, whereas it was elevated in the si-DsbA-L+RSG group (Fig. 4e).

4 Discussion

The enzyme DsbA-L was firstly found and named by Liu et al. (2008); they identified that DsbA-L could regulate the multimerization of adiponectin. Since

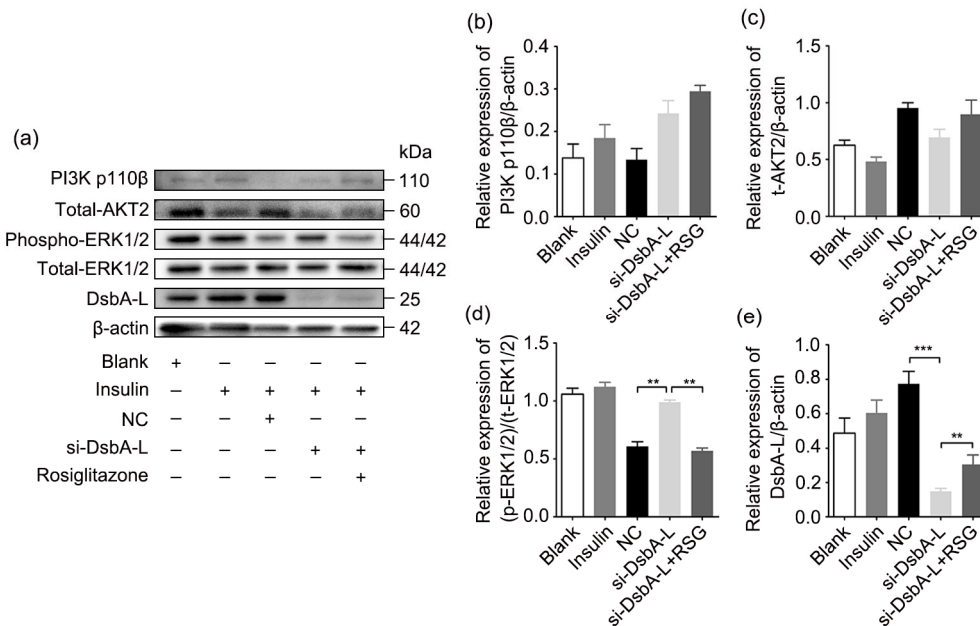


Fig. 4 Effect of si-DsbA-L on action of PPAR γ agonist of improving insulin resistance

(a) Representative image of the immunoblots of cell samples (20 μ g); (b) Relative expression of PI3K p110 β quantified with β -actin; (c) Relative expression of total-AKT2 quantified with β -actin; (d) Relative expression of phospho-ERK1/2 quantified with total-ERK1/2; (e) Relative expression of DsbA-L quantified with β -actin. PPAR γ : peroxisome proliferator-activated receptor γ ; si-DsbA-L: small interfering RNA (siRNA) of disulfide-bond A oxidoreductase-like protein (DsbA-L); PI3K: phosphatidylinositol 3-kinase; AKT2: protein kinase B (PKB) β ; ERK1/2: extracellular signal-regulated kinase 1/2; NC: negative control; RSG: rosiglitazone; t-AKT2: total-AKT2; p-ERK1/2: phospho-ERK1/2; t-ERK1/2: total-ERK1/2. Data are shown as mean $\pm$ standard deviation (SD) ($n=3$). ** $P<0.01$, *** $P<0.001$

then, DsbA-L has appeared in front of the public as a multifaceted player in adiponectin secretion (Wang and Scherer, 2008). It is of great significance to elaborate how this protein participates in the pathophysiological processes of GDM. In the present study, we found that the level of DsbA-L was significantly reduced in GDM-affected adipose tissue. Previous reports revealed that patients with obesity had a significantly decreased level of DsbA-L in contrast to normal-weight controls (Chen et al., 2017). Women with GDM are potentially associated with obesity, and most cases of GDM are related to increased BMI (Kim et al., 2010; Giannakou et al., 2019). This, to some extent, explains the low level of DsbA-L in GDM.

It was observed in previous studies that DsbA-L overexpression could improve insulin sensitivity in mice by promoting the phosphorylation of adenosine 5'-monophosphate-activated protein kinase (AMPK) and PKB/AKT (Liu et al., 2012). Adiponectin signaling primarily enhances insulin receptor substrates (IRSs) to sensitize insulin action (Achari and Jain, 2017). Promoting the biosynthesis and secretion of

adiponectin may be an effective way to treat metabolic diseases (Liu and Liu, 2012). The multimerization of adiponectin is attributed to DsbA-L, which seems to suggest a potential relationship between DsbA-L and insulin sensitivity.

Adiponectin multimerization was shown to be promoted by DsbA-L through the regulation of disulfide bond formation (Achari and Jain, 2017). A study demonstrated that circulating levels of adiponectin were reduced in obesity and associated diseases (Liu et al., 2008), indicating that adiponectin was involved in the pathophysiology of these diseases. In fact, adiponectin has some beneficial properties, such as anti-inflammatory and insulin sensitization (Simpson and Whitehead, 2010). Increasing the levels of DsbA-L could improve the stability of adiponectin by inhibiting the adverse effect of ER stress (Zhou et al., 2010). Therefore, heightening the expression of DsbA-L is likely to be a promising strategy to increase insulin sensitivity (Zhou et al., 2010).

In the present study, the expression of DsbA-L was downregulated, resulting in decreased PPAR γ agonist function, suggesting that DsbA-L not only

participates in insulin sensitivity, but also plays a critical role in insulin signaling pathways. Several studies have indicated extensively that DsbA-L is found downstream of PPAR γ (Jin et al., 2015; He et al., 2016). Consistently with these findings and furthermore, we conclude that DsbA-L is extremely significant in PPAR γ action. The latter may exert an effect on the PI3K-AKT pathway by regulating the transcription of DsbA-L. The molecular process by which PPAR γ agonists improve insulin resistance was further elaborated in mechanism.

Insulin resistance is associated with a damaged insulin signaling pathway (Plows et al., 2018). Phosphorylation of IRS is accompanied by the activation of two insulin signaling pathways: the PI3K-AKT pathway and the ras-MAPK pathway (Taniguchi et al., 2006). There are different negative and positive feedback loops in the AKT and MAPK pathways at the molecular level (Arkun, 2016). The PI3K-AKT signaling pathway is important in the metabolic role of insulin. In this pathway, the p110 β subtype of PI3K is essential for mediating the glucose uptake of insulin sensitive tissues. Differently from AKT1 and AKT3, AKT2 is particularly enriched in the liver and adipose tissue, and is co-located with glucose transporter 4 (GLUT4), which is responsible for bringing glucose into the cell as energy supply (Plows et al., 2018). Interestingly, DsbA-L suppression stimulated the phosphorylation of ERK1/2, which might be attributed to mitochondrial dysfunction. The ERK1/2 of the MAPK pathway is mainly involved in the regulation of cell growth and survival, but not the metabolic effect of insulin. As commonly known, mitochondria provide energy for vital cell functions. Therefore, when mitochondrial function is injured via *DsbA-L* silencing, cells promote the activation of ERK1/2 signaling in order to adapt to new energy requirements and repair the damage (Pal et al., 2020).

We also showed that chemerin could elevate the expression of DsbA-L, which might further increase adiponectin levels. Although we did not verify this possibility via experiments, recent research supports that chemerin exerts an effect on adiponectin to regulate fat metabolism (Ferland et al., 2020), which is attributed to the interactions of adipocytokines. It has been reported that tumor necrosis factor- α (TNF- α) inhibits the multimerization and secretion of adiponectin by impairing DsbA-L (He et al., 2016).

Chemerin may be a favorable adipokine, as its function is unlike that of TNF- α . Chemerin was originally discovered as an inflammatory chemokine, encoded by the retinoic acid receptor responder 2 (*RARRES2*) gene. Further studies have shown that it is a novel adipocytokine secreted by adipose tissue, and is involved in the pathophysiological mechanism of numerous diseases, including obesity and metabolic syndrome (Goralski et al., 2007; Helfer and Wu, 2018). In addition to that of adiponectin, the understanding of the regulatory function of DsbA-L and its interaction with other adipokines is incomplete (Liu et al., 2012). Further research is required to elucidate these mechanisms.

It should be noted that our research has some limitations. Neither the expression of GLUT4 downstream of the insulin signaling pathway is verified by our study, nor does it be clear whether HTR-8/SVneo cells cultured with high glucose in vitro are similar to the circulatory blood glucose levels of GDM patients. The role of DsbA-L in the action of PPAR γ agonists in vivo remains to be further explored in animal experiments.

5 Conclusions

In summary, we confirmed the necessity of DsbA-L in PPAR γ agonist function that improves insulin resistance, and for the first time we demonstrated that chemerin promotes DsbA-L expression.

Contributors

Xuan ZHOU performed the experiments and prepared this manuscript. Jia-qi LI, Li-jie WEI, Jing JIA and Jing-yi ZHANG participated in carrying out the experiment. Xuan ZHOU and Jia-qi LI conducted data analysis and discussed the results. Meng-zhou HE and Li-jie WEI reviewed and edited the manuscript. Ling FENG and Shao-shuai WANG designed, organized, and supervised the project. All authors have read and approved this manuscript. Therefore, they have full access to all data in this study and take responsibility for the integrity and security of these data.

Acknowledgments

We thank the Obstetric Laboratory of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China for providing the experimental platform.

Compliance with ethics guidelines

Xuan ZHOU, Jia-qi LI, Li-jie WEI, Meng-zhou HE, Jing JIA, Jing-yi ZHANG, Shao-shuai WANG and Ling FENG declare that they have no conflict of interest.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5). Informed consent was obtained from all patients for being included in the study.

References

- Achari AE, Jain SK, 2017. Adiponectin, a therapeutic target for obesity, diabetes, and endothelial dysfunction. *Int J Mol Sci*, 18(6):1321.
<https://doi.org/10.3390/ijms18061321>
- Arkun Y, 2016. Dynamic modeling and analysis of the cross-talk between insulin/AKT and MAPK/ERK signaling pathways. *PLoS ONE*, 11(3):e0149684.
<https://doi.org/10.1371/journal.pone.0149684>
- Bai JL, Cervantes C, Liu J, et al., 2017. DsbA-L prevents obesity-induced inflammation and insulin resistance by suppressing the mtDNA release-activated cGAS-cGAMP-STING pathway. *Proc Natl Acad Sci USA*, 114(46):12196-12201.
<https://doi.org/10.1073/pnas.1708744114>
- Basak S, Das MK, Srinivas V, et al., 2015. The interplay between glucose and fatty acids on tube formation and fatty acid uptake in the first trimester trophoblast cells, HTR8/SVneo. *Mol Cell Biochem*, 401(1-2):11-19.
<https://doi.org/10.1007/s11010-014-2287-9>
- Chen HZ, Bai JL, Dong F, et al., 2017. Hepatic DsbA-L protects mice from diet-induced hepatosteatosis and insulin resistance. *FASEB J*, 31(6):2314-2326.
<https://doi.org/10.1096/fj.201600985R>
- Deng X, Yang G, Zheng X, et al., 2020. Plasma mtDNA copy numbers are associated with *GSTK1* expression and inflammation in type 2 diabetes. *Diabet Med*, 37(11):1874-1878.
<https://doi.org/10.1111/dme.14132>
- Ferland DJ, Garver H, Contreras GA, et al., 2020. Chemerin contributes to *in vivo* adipogenesis in a location-specific manner. *PLoS ONE*, 15(2):e0229251.
<https://doi.org/10.1371/journal.pone.0229251>
- Gao F, Fang QC, Zhang R, et al., 2009. Polymorphism of DsbA-L gene associates with insulin secretion and body fat distribution in Chinese population. *Endocr J*, 56(3):487-494.
<https://doi.org/10.1507/endocrj.k08e-322>
- Giannakou K, Evangelou E, Yiallourous P, et al., 2019. Risk factors for gestational diabetes: an umbrella review of meta-analyses of observational studies. *PLoS ONE*, 14(4):e0215372.
<https://doi.org/10.1371/journal.pone.0215372>
- Goralski KB, McCarthy TC, Hanniman EA, et al., 2007. Chemerin, a novel adipokine that regulates adipogenesis and adipocyte metabolism. *J Biol Chem*, 282(38):28175-28188.
<https://doi.org/10.1074/jbc.M700793200>
- Griffith RJ, Alsweiler J, Moore AE, et al., 2020. Interventions to prevent women from developing gestational diabetes mellitus: an overview of Cochrane Reviews. *Cochrane Database Syst Rev*, 6(6):CD012394.
<https://doi.org/10.1002/14651858.CD012394.pub3>
- He YD, Lu LF, Wei X, et al., 2016. The multimerization and secretion of adiponectin are regulated by TNF- α . *Endocrine*, 51(3):456-468.
<https://doi.org/10.1007/s12020-015-0741-4>
- Helfer G, Wu QF, 2018. Chemerin: a multifaceted adipokine involved in metabolic disorders. *J Endocrinol*, 238(2):R79-R94.
<https://doi.org/10.1530/JOE-18-0174>
- Jin D, Sun J, Huang J, et al., 2015. Peroxisome proliferator-activated receptor γ enhances adiponectin secretion via up-regulating DsbA-L expression. *Mol Cell Endocrinol*, 411:97-104.
<https://doi.org/10.1016/j.mce.2015.04.015>
- Kim SY, England L, Wilson HG, et al., 2010. Percentage of gestational diabetes mellitus attributable to overweight and obesity. *Am J Public Health*, 100(6):1047-1052.
<https://doi.org/10.2105/AJPH.2009.172890>
- Kuricova K, Pácal L, Šoupal J, et al., 2016. Effect of glucose variability on pathways associated with glucotoxicity in diabetes: evaluation of a novel *in vitro* experimental approach. *Diabetes Res Clin Pract*, 114:1-8.
<https://doi.org/10.1016/j.diabres.2016.02.006>
- Lampropoulou E, Lymperopoulou A, Charonis A, 2016. Reduced expression of ERp46 under diabetic conditions in β -cells and the effect of liraglutide. *Metabolism*, 65(1):7-15.
<https://doi.org/10.1016/j.metabol.2015.09.011>
- Lebovitz HE, 2019. Thiazolidinediones: the forgotten diabetes medications. *Curr Diab Rep*, 19(12):151.
<https://doi.org/10.1007/s11892-019-1270-y>
- Li ZY, Cheng YJ, Wang DY, et al., 2020. Incidence rate of type 2 diabetes mellitus after gestational diabetes mellitus: a systematic review and meta-analysis of 170,139 women. *J Diabetes Res*, 2020:3076463.
<https://doi.org/10.1155/2020/3076463>
- Liu ML, Liu F, 2012. Up- and down-regulation of adiponectin expression and multimerization: mechanisms and therapeutic implication. *Biochimie*, 94(10):2126-2130.
<https://doi.org/10.1016/j.biochi.2012.01.008>
- Liu ML, Zhou LJ, Xu AM, et al., 2008. A disulfide-bond A oxidoreductase-like protein (DsbA-L) regulates adiponectin multimerization. *Proc Natl Acad Sci USA*, 105(47):18302-18307.
<https://doi.org/10.1073/pnas.0806341105>
- Liu ML, Xiang RH, Wilk SA, et al., 2012. Fat-specific DsbA-L overexpression promotes adiponectin multimerization and protects mice from diet-induced obesity and insulin resistance. *Diabetes*, 61(11):2776-2786.

- <https://doi.org/10.2337/db12-0169>
- Liu ML, Chen HZ, Wei L, et al., 2015. Endoplasmic reticulum (ER) localization is critical for DsbA-L protein to suppress ER stress and adiponectin down-regulation in adipocytes. *J Biol Chem*, 290(16):10143-10148. <https://doi.org/10.1074/jbc.M115.645416>
- Lorenzo-Almorós A, Hang T, Peiró C, et al., 2019. Predictive and diagnostic biomarkers for gestational diabetes and its associated metabolic and cardiovascular diseases. *Cardiovasc Diabetol*, 18:140. <https://doi.org/10.1186/s12933-019-0935-9>
- Pal S, Rao GN, Pal A, 2020. High glucose-induced ROS accumulation is a critical regulator of ERK1/2-Akt-tuberin-mTOR signalling in RGC-5 cells. *Life Sci*, 256:117914. <https://doi.org/10.1016/j.lfs.2020.117914>
- Plows JF, Stanley JL, Baker PN, et al., 2018. The pathophysiology of gestational diabetes mellitus. *Int J Mol Sci*, 19(11):3342. <https://doi.org/10.3390/ijms19113342>
- Simpson F, Whitehead JP, 2010. Adiponectin—it's all about the modifications. *Int J Biochem Cell Biol*, 42(6):785-788. <https://doi.org/10.1016/j.biocel.2009.12.021>
- Taniguchi CM, Emanuelli B, Kahn CR, 2006. Critical nodes in signalling pathways: insights into insulin action. *Nat Rev Mol Cell Biol*, 7(2):85-96. <https://doi.org/10.1038/nrm1837>
- Wang XY, Pan JY, Liu H, et al., 2019. AIM2 gene silencing attenuates diabetic cardiomyopathy in type 2 diabetic rat model. *Life Sci*, 221:249-258. <https://doi.org/10.1016/j.lfs.2019.02.035>
- Wang ZV, Scherer PE, 2008. DsbA-L is a versatile player in adiponectin secretion. *Proc Natl Acad Sci USA*, 105(47):18077-18078. <https://doi.org/10.1073/pnas.0810027105>
- Zhou LJ, Liu ML, Zhang JJ, et al., 2010. DsbA-L alleviates endoplasmic reticulum stress-induced adiponectin down-regulation. *Diabetes*, 59(11):2809-2816. <https://doi.org/10.2337/db10-0412>
- Zhou X, Wei LJ, Li JQ, et al., 2020. The activation of peroxisome proliferator-activated receptor γ enhances in-

sulin signaling pathways via up-regulating chemerin expression in high glucose treated HTR-8/SVneo cells. *Matern-Fetal Med*, 2(3):131-140.

<https://doi.org/10.1097/FM9.0000000000000044>

中文概要

题目：高糖细胞模型中 *DsbA-L* 基因沉默损害 PPAR γ 激动剂改善胰岛素抵抗的功能

目的：在高糖滋养细胞模型中，探讨二硫键 A 氧化还原酶样蛋白（DsbA-L）在过氧化物酶体增殖物激活受体 γ （PPAR γ ）激动剂改善胰岛素抵抗过程中的作用，以及 DsbA-L 与趋化素（chemerin）的关系。

创新点：发现 DsbA-L 在 PPAR γ 激动剂改善胰岛素抵抗的过程中起作用，并首次证明 chemerin 能促进 DsbA-L 的表达水平。

方法：采用免疫组织化学（IHC）和蛋白免疫印迹法（western blot）检测妊娠期糖尿病（GDM）患者与正常对照组孕妇的皮下脂肪组织中 DsbA-L 的定位和表达差异。在高糖滋养细胞模型中，通过 western blot 研究 PPAR γ 激动剂和 chemerin 对 DsbA-L 蛋白的调节作用，并探究 *DsbA-L* 基因沉默对 PPAR γ 激动剂调控胰岛素信号通路分子磷脂酰肌醇 3 激酶（PI3K）、蛋白激酶 B（PKB/AKT）和细胞外信号调节激酶 1/2（ERK1/2）蛋白表达的影响。

结论：GDM 患者的皮下脂肪组织中 DsbA-L 的水平较对照组低。PPAR γ 激动剂和 chemerin 均可增强 DsbA-L 蛋白的表达。*DsbA-L* 基因沉默影响 PPAR γ 激动剂对胰岛素信号 PI3K-AKT 通路的上调作用。

关键词：二硫键 A 氧化还原酶样蛋白（DsbA-L）；过氧化物酶体增殖物激活受体 γ （PPAR γ ）；趋化素（Chemerin）；胰岛素信号通路；妊娠期糖尿病