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THERAPY

Exercise Reduces Insulin Resistance in Type 2 Diabetes Mellitus via Mediating the lncRNA MALAT1/MicroRNA-382-3p/Resistin Axis

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Insulin resistance (IR) is the primary pathological mechanism underlying type 2 diabetes mellitus (T2DM). Here, the study aimed to ascertain whether and how exercise mediates IR in T2DM. An *in vivo* mouse model of high-fat diet-induced IR and an *in vitro* high-glucose-induced IR model were constructed. High long non-coding RNA (lncRNA) metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) expression was detected in T2MD and was positively correlated with HOMA-IR and resistin levels. Then, short hairpin RNA targeting MALAT1 (sh-MALAT1) or pcDNA-MALAT1 was delivered into human umbilical vein endothelial cells (HUVECs) to knock down or upregulate its expression, respectively. Silencing of MALAT1 resulted in reduced levels of resistin, Ang II, tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1), endothelin-1 (ET-1), and p-insulin receptor substrate-1 (p-IRS)/ISR-1, and decreased cell migration, as well as enhanced glucose uptake and levels of nitric oxide (NO) and p-Akt/Akt. In the IR mouse model, exercise was observed to downregulate MALAT1 to reduce resistin, whereby exercise reduced homeostatic model assessment-insulin resistance (HOMA-IR). Besides, exercise also elevated microRNA-382-3p (miR-382-3p) expression in the serum of IR mice. Dual-luciferase reporter and RNA binding protein immunoprecipitation (RIP) assays identified that MALAT1 could bind to miR-382-3p to upregulate resistin. Collectively, the key observations of the study provide evidence that inhibition of MALAT1 elevates miR-382-3p to repress resistin, which consequently underlies the mechanism of exercise protecting against IR, highlighting a direction for T2DM therapy development.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by reduced insulin action, elevated hepatic glucose production, and impaired insulin secretory function.[1] Globally, approximately 285 million people have been reported to be affected by T2DM.[2] Over the past decade, the incidence of T2DM has been growing due to the increasing prevalence of obesity.[3] Insulin resistance (IR) induced by obesity is a major determinant associated with metabolic syndrome that precedes the progression of T2DM

and is thus a driver behind the emerging diabetes epidemic.[4] IR is a pathological condition in which cells are unable to efficiently respond to stimulation by insulin.[5] Reduction of IR can result in the alleviation of T2DM.[6] Interestingly, obese participants that underwent a controlled 12-week aerobic exercise program benefited from diminished visceral and hepatic fat accumulation, as well as inhibited IR.[7] In particular, swimming was shown to suppress high-fat diet (HFD)-induced IR by decreasing liver and muscle lipid accumulation and modulating energy metabolism in skeletal muscles.[8] An in-depth understanding of the molecular mechanism of exercise conferred for its protective effect against IR may aid in the development of novel therapeutic options for T2DM.

Long non-coding RNAs (lncRNAs), which include over 200 nucleotides, have emerged as mediators to exert an effect on IR in T2DM[9] and are involved in diverse pathological processes in many human diseases through regulating gene expression.[10] For instance, lncRNA FR030200 and lncRNA FR402720 are reported to be highly expressed in HFD-induced IR and significantly repressed after long-term exercise training.[11] Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), also known as nuclear-enriched abundant transcript 2 (NEAT2), is defined as a bona fide lncRNA located in nuclear bodies and widely expressed in human tissues.[12,13] Silencing of MALAT1 is considered to be a therapeutic strategy for microvascular complications related to diabetes.[14] lncRNA MALAT1 is reported to act as a competing endogenous RNA (ceRNA) of microRNA-142-3p (miR-142-3p) and consequently regulates mRNA translation of its target gene HMGA2.[15] It is known that miRNAs lead to aberrant gene expression in insulin-resistant tissues, which is implicated in the progression of diabetes.[16] For example, elevation of miR-145 by resistin is shown to inhibit glucose uptake in HepG2 cells and induce IR in hepatocytes.[17] Resistin is a protein hormone released by adipocytes, which contributes to IR and purportedly acts as a link between obesity and diabetes.[18] Furthermore, resistin is reported to promote cancer cell
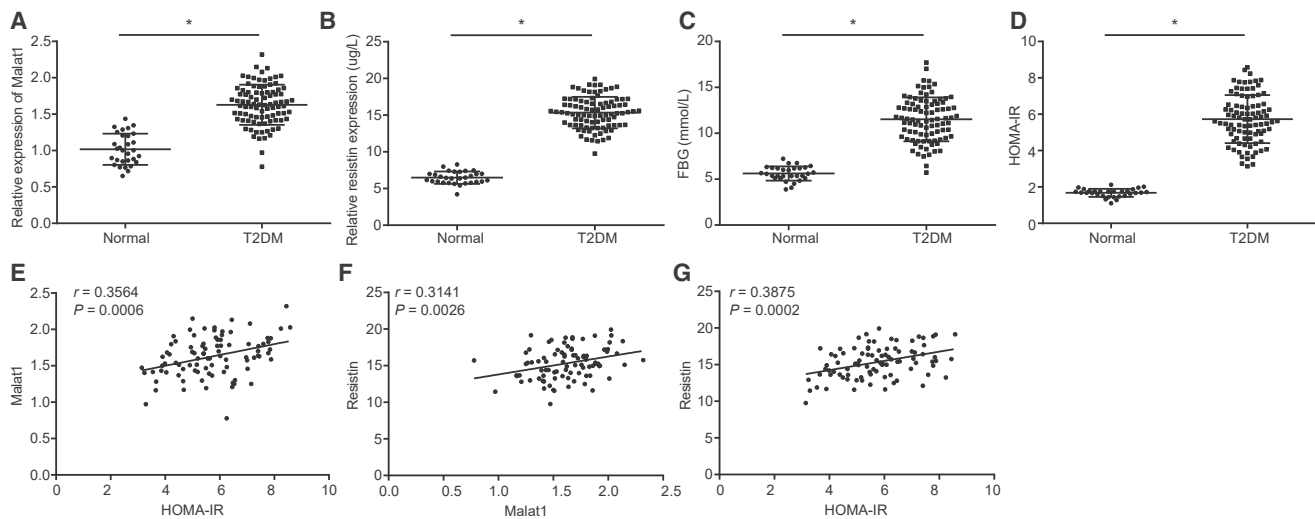
Figure 1. MALAT1 Is Highly Expressed in Patients with T2DM and Positively Correlates to HOMA-IR and Resistin Levels

(A) MALAT1 expression in serum from healthy individuals and T2DM patients determined using qRT-PCR. (B) Serum resistin level from healthy individuals and T2DM patients measured using ELISA. (C) FBG in serum from healthy individuals and T2DM patients detected by a biochemical analyzer. (D) HOMA-IR level in serum from healthy individuals and T2DM patients. (E) Pearson's correlation analysis of MALAT1 expression with HOMA-IR level. (F) Pearson's correlation analysis of MALAT1 expression and resistin level. (G) Pearson's correlation analysis of resistin level and HOMA-IR level; *$p < 0.05$, versus healthy individuals; $n = 30$ in healthy individuals; $n = 90$ in T2DM patients; measurement data were depicted as mean ± SD; comparisons between two groups were analyzed using unpaired t test.

adhesion to human umbilical vein endothelial cells (HUVECs).[19,20] The aim of the current study was to investigate the potential inhibitory role of exercise in IR-induced animal models and to ascertain whether exercise functions by regulating MALAT1, miR-382-3p, and resistin. An IR HUVEC model was further applied to identify the relationship among MALAT1, miR-382-3p, and resistin.

RESULTS

MALAT1 Is Upregulated in Patients with T2DM and Positively Correlates with HOMA-IR and Resistin

Initially, the expression of MALAT1 in serum from healthy individuals and T2DM patients was determined using qRT-PCR, which indicated that MALAT1 expression was increased in T2DM patients as compared with healthy individuals ($p < 0.05$) (Figure 1A). Subsequently, ELISA results displayed increased serum levels of resistin in T2DM patients as compared to those in healthy individuals ($p < 0.05$) (Figure 1B). Next, as measured by a biochemical analyzer, the level of fasting blood glucose (FBG) in T2DM patients was higher than that in healthy individuals ($p < 0.05$) (Figure 1C). Homeostatic model assessment-insulin resistance (HOMA-IR) was calculated based on the formula: fasting insulin (FINS) (mIU/L) $\times$ FBG (mmol/L)/22.5, which revealed that the T2DM patients showed an elevated IR level as compared to healthy individuals ($p < 0.05$) (Figure 1D). Subsequent correlation analyses of MALAT1 with HOMA-IR and resistin levels suggested that when MALAT1 expression was decreased, the levels of HOMA-IR and resistin were also reduced; meanwhile, once resistin level was reduced, HOMA-IR level was decreased ($p < 0.05$; Figures 1E–1G). Overall, it was demonstrated that MALAT1 was upregulated in patients with T2DM. Besides, MALAT1 expression was positively correlated with HOMA-IR and

resistin levels and resistin was also in a positive correlation with HOMA-IR in T2DM.

Exercise Alleviates IR by Reducing MALAT1 and Resistin Levels

Following the confirmation in regards to expression of MALAT1 in T2DM and the correlations of MALAT1 with HOMA-IR and resistin, the attention and focus in the experiment were shifted to elucidate whether decreased MALAT1 could ameliorate IR and whether it was associated with the IR-reducing effect exerted by exercise. To start, after fed with HFD, HOMA-IR and resistin levels were increased (Figures 2A and 2B). Next, the mice from the IR model experienced exercise or treated with sh-MALAT1. The results indicated that IR mice either experienced exercise or treated with short hairpin RNA targeting MALAT1 (sh-MALAT1) presented with reduced HOMA-IR (all $p < 0.05$; Figure 2A). Subsequently, ELISA revealed that resistin level was reduced in IR mice after exercise or sh-MALAT1 treatment (both $p < 0.05$) (Figure 2B). These results indicated that exercise or MALAT1 silencing could reduce resistin level of IR mice. Next, qRT-PCR was applied to determine the expression of MALAT1 in serum of mice fed a normal diet or HFD, which suggested that MALAT1 expression was increased in IR mice as compared with mice fed with a normal diet ($p < 0.05$), but it was decreased in IR mice after exercise or MALAT1 silencing (all $p < 0.05$) (Figure 2C). These results suggested that exercise could ameliorate IR by inhibiting the levels of MALAT1 and resistin.

Silencing of MALAT1 Restrains Resistin Level to Alleviate IR of HUVECs

To elucidate the potential mechanisms of MALAT1 in IR, HUVECs were cultured with high glucose (HG) medium and stimulated with
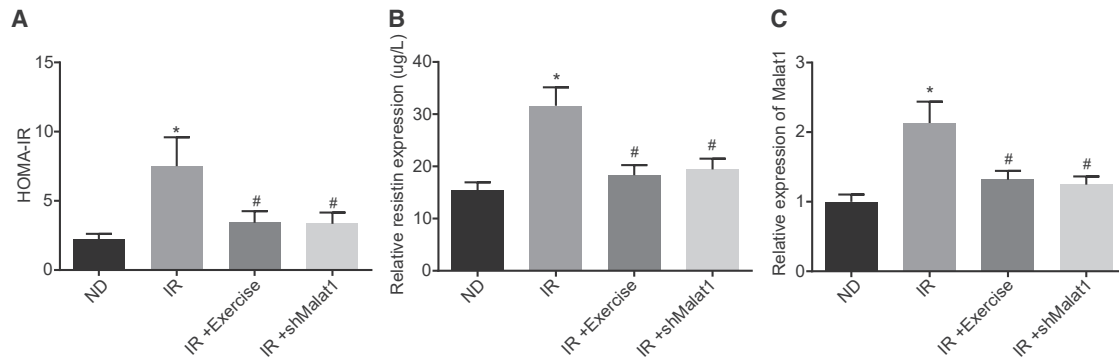
Figure 2. Exercise Ameliorates IR by Inhibiting MALAT1 and Resistin Levels

(A) HOMA-IR level in serum of IR mice subjected to exercise or IR mice treated with sh-MALAT1. (B) Resistin level in serum of IR mice subjected to exercise or IR mice treated with sh-MALAT1 measured using ELISA. (C) MALAT1 expression in serum of IR mice subjected to exercise or IR mice treated with sh-MALAT1 determined using qRT-PCR; *$p < 0.05$, versus mice fed with a normal diet; #$p < 0.05$, versus IR mice; measurement data were depicted as mean ± SD; comparisons among multiple groups were analyzed using one-way ANOVA; n = 10.

insulin to establish HUVEC IR model. Then qRT-PCR revealed that MALAT1 expression was increased in IR HUVECs but decreased after transfection with sh-MALAT1 ($p < 0.05$) (Figure 3A). Western blot analysis results showed that resistin expression was increased in IR HUVECs, which was partially blocked upon sh-MALAT1 transfection ($p < 0.05$) (Figure 3B). Moreover, glucose uptake was reduced in IR HUVECs, while silencing MALAT1 resulted in increased glucose uptake ($p < 0.05$) (Figure 3C). Subsequent results obtained from a Transwell assay revealed that the migration of IR HUVECs was decreased, whereas in sh-MALAT1-transfected cells, the invasion ability was inhibited ($p < 0.05$) (Figure 3D). ELISA presented that IR HUVECs showed a decreased level of nitric oxide (NO) and elevated levels of Ang II, tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), soluble intercellular adhesion molecule-1 (sICAM-1), and soluble vascular cell adhesion molecule-1 (sVCAM-1); however, those changes in IR HUVECs were all reversed by sh-MALAT1 (all $p < 0.05$) (Figures 3E and 3F). In addition, the protein expression of endothelin-1 (ET-1), p-insulin receptor substrate-1 (IRS) (Ser), IRS-1, p-Akt, and Akt was measured using western blot analysis, which indicated that IR HUVECs displayed increased protein expression of ET-1 and p-IRS/IRS-1 and decreased p-Akt/Akt protein expression, whereas opposite changes in the aforementioned factors were observed after silencing MALAT1 (Figure 3G). Therefore, knockdown of MALAT1 could inhibit resistin expression and cell migration but promote glucose uptake so as to relieve IR-induced vascular endothelial injury. Altogether, silencing of MALAT1 could alleviate IR by suppressing resistin expression.

Silencing of MATAT1 Upregulates miR-382-3p to Inhibit Resistin Expression

Following the results that silencing of MALAT1 contributed to the alleviation of IR, the miRNAs that regulate Retn (resistin) were predicted, and subsequently three mouse miRNAs were obtained, namely, miR-382-3p, miR-6974-3p, and miR-8097 (Figure 4A). miR-382-3p was selected as the candidate miRNA. miR-382-3p was highly conserved between mice and humans (Figure 4B). Based on

the DIANA and starBase databases, we found a binding site between MALAT1 and miR-382-3p (Figure 4C). Dual-luciferase reporter assay was utilized to confirm the relationship between MALAT1 and miR-382-3p, or between miR-382-3p and resistin, which displayed that luciferase activity of pGL3-MALAT1 or pGL3-resistin-3′UTR was reduced in cells co-transfected with miR-382-3p mimic ($p < 0.05$), and the luciferase activity of pGL3-resistin-3′UTR was also reduced in cells co-transfected with sh-MALAT1 ($p < 0.05$) (Figure 4D). These findings suggested that MALAT1 could bind to miR-382-3p and mediated resistin expression. qRT-PCR results also revealed that HUVECs transfected with miR-382-3p mimic showed reduced expression of MALAT1 and resistin (both $p < 0.05$) (Figure 4E), and western blot and qRT-PCR analyses displayed that transfection of sh-MALAT1 or miR-382-3p mimic in HUVECs resulted in inhibited resistin expression (both $p < 0.05$; Figure 4F). Furthermore, overexpressing MALAT1 in HUVECs upregulated resistin expression, which could be blocked by co-transfection with miR-382-3p mimic and pcDNA-MALAT1 (both $p < 0.05$; Figure 4F). Finally, RNA binding protein immunoprecipitation (RIP) assay results displayed that both MALAT1 and miR-382-3p bound to Argonaute2 (Ago2) ($p < 0.05$) (Figure 4G). The obtained data suggested that MATAT1 could increase resistin expression by competitively binding to miR-382-3p.

Exercise Alleviates IR by Promoting miR-382-3p Expression

Because MALAT1 could bind to miR-382-3p to regulate resistin, we decided to further investigate whether exercise regulates miR-382-3p, thus mediating IR in T2DM. The results of qRT-PCR demonstrated the miR-382-3p was expressed at a lower level in the serum of patients with T2DM than in the serum of healthy controls ($p < 0.05$) (Figure 5A). Besides, IR mice showed decreased miR-382-3p expression in serum when compared to mice fed with a normal diet, whereas IR mice after exercise displayed elevated miR-382-3p expression in serum (both $p < 0.05$) (Figure 5B). Taken together, the results indicated that exercise could elevate miR-382-3p expression and consequently alleviate IR.
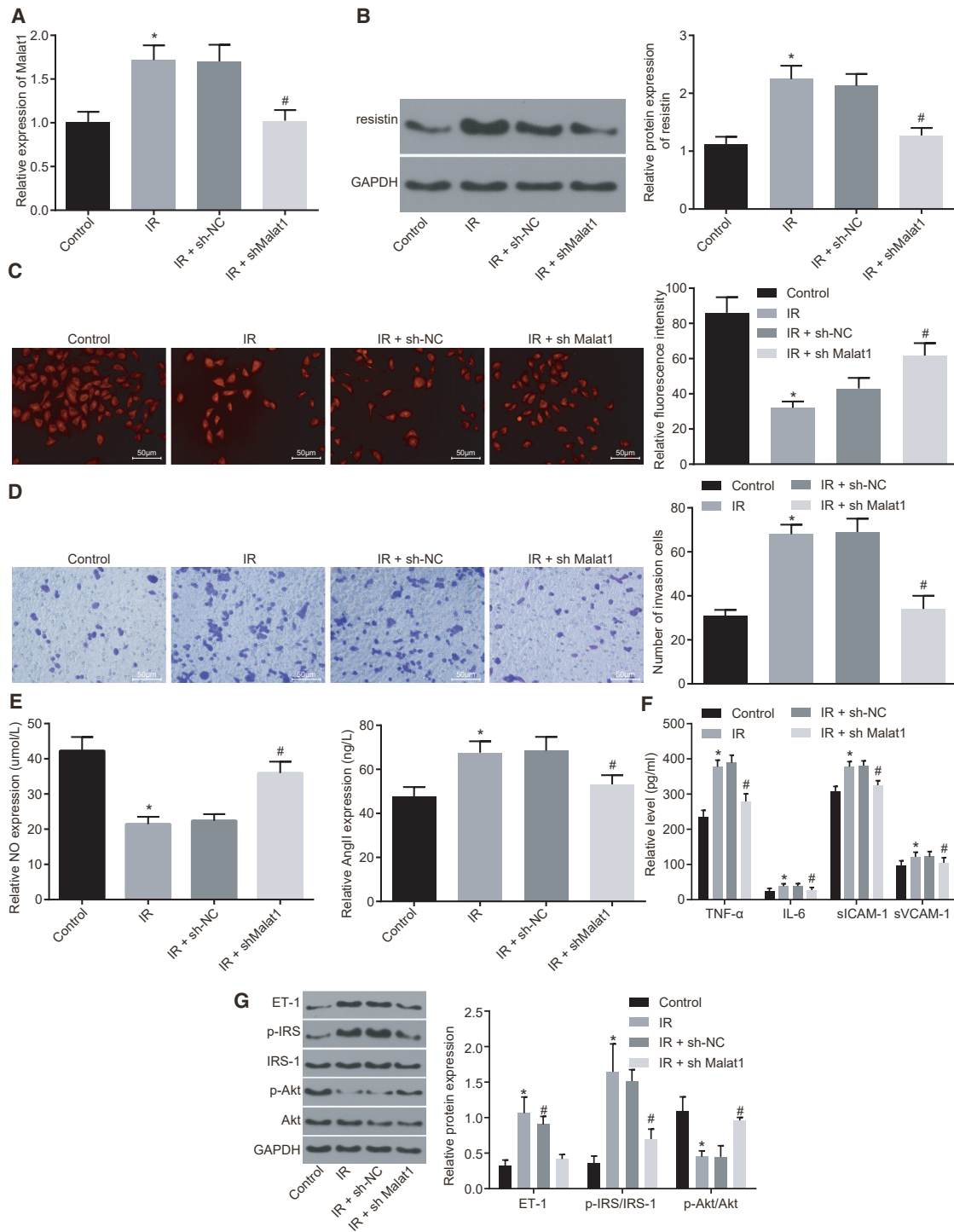
Figure 3. Silencing of MALAT1 Alleviates IR in HUVECs by Suppressing Resistin Expression

(A) MALAT1 expression in IR HUVECs determined using qRT-PCR. (B) Western blot analysis of resistin level in IR HUVECs. (C) Glucose uptake in IR HUVECs (200×). (D) Migration of IR HUVECs detected using Transwell assay (200×). (E) serum levels of NO and Ang II in supernatant of IR HUVECs measured using ELISA. (F) Serum levels of TNF-α, IL-6, sICAM-1, and sVCAM-1 in supernatant of IR HUVECs measured using ELISA. (G) Western blot analysis of ET-1, p-IRS (Ser), ISR-1, p-Akt, and Akt proteins in IR HUVECs; *p < 0.05, versus cells cultured with DMEM supplemented with 5.5 mmol/L glucose (control); #p < 0.05, versus cells cultured with DMEM supplemented with 33 mmol/L glucose and transfected with sh-NC (IR +sh-NC); measurement data were depicted as mean ± SD; comparisons among multiple groups were analyzed by one-way ANOVA; the experiment was repeated three times.
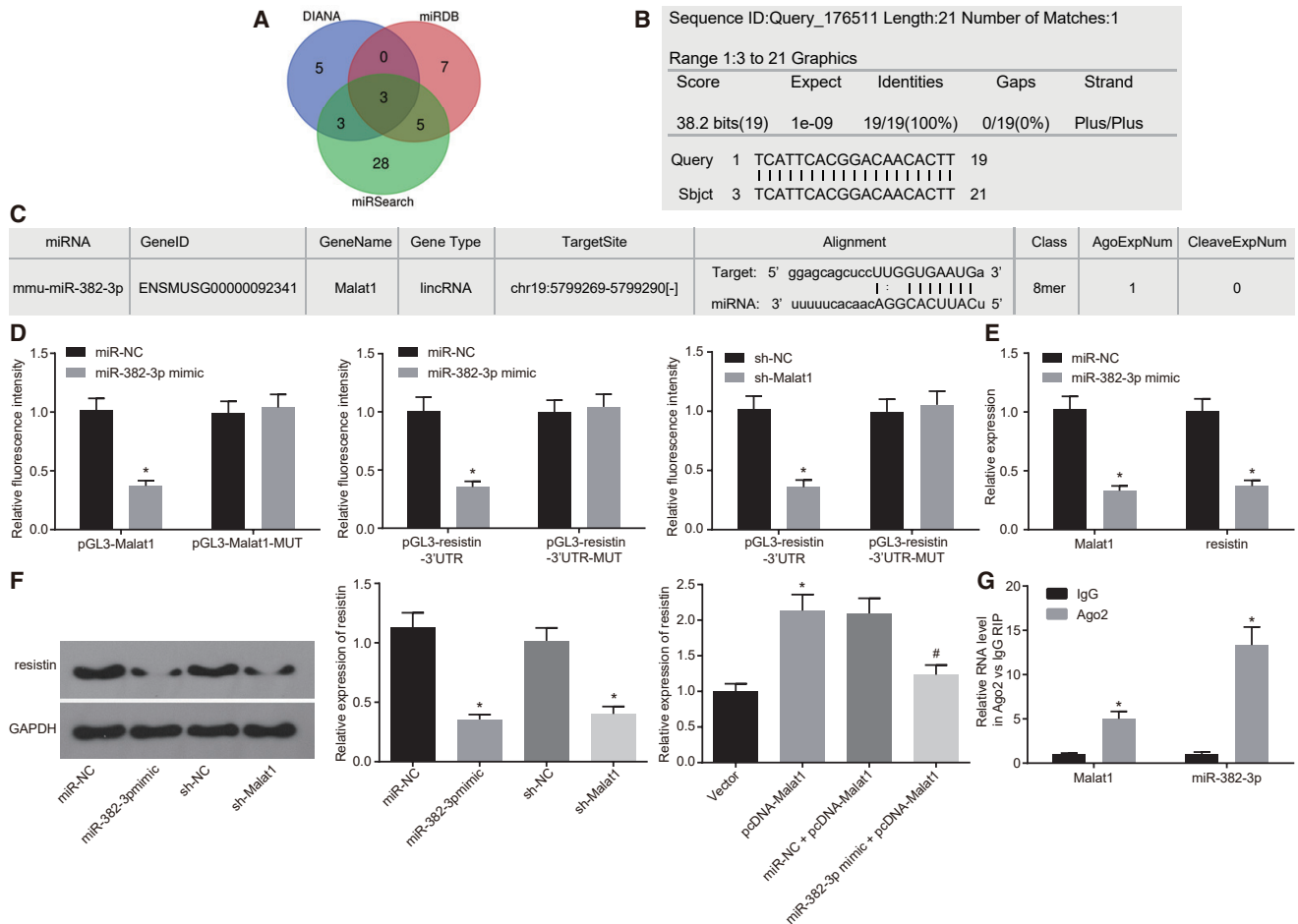
Figure 4. Silencing of MATAT1 Upregulates miR-382-3p to Inhibit Resistin Expression

(A) Putative miRNAs regulating resistin predicted by miRDB, miRSearch and DIANA. (B) Comparison of sequence homology of miR-382-3p in mice and human beings using BLAST. (C) Potential binding sites between MALAT1 and miR-382-3p predicted by the DIANA and starBase databases. (D) Relationship among MALAT1, miR-382-3p, and resistin confirmed by dual-luciferase reporter assay. (E) Expression of MALAT1 and resistin in response to transfection with miR-382-3p mimic determined using qRT-PCR. (F) Resistin expression in response to transfection with miR-382-3p mimic or sh-MALAT1, and/or pcDNA-MALAT1 detected using western blot analysis and qRT-PCR. (G) Correlation of MALAT1 and miR-382-3p with Ago2 using RIP assay; *$p < 0.05$, versus cells transfected with miR-NC, sh-NC, IgG, or vector plasmids; #$p < 0.05$, versus cells co-transfected with miR-NC and pcDNA-MALAT1 plasmids; measurement data were depicted as mean ± SD; comparisons between two groups were analyzed using unpaired t test; comparisons among multiple groups were analyzed by one-way ANOVA; the experiment was repeated three times.

MALAT1 Increases IR in HUVECs by Impairing miR-382-3p-Mediated Degradation of Resistin

At last, in order to clarify the involvement of MALAT1/miR-382-3p/resistin axis in IR, HUVECs from the *in vitro* IR model were transfected with pcDNA-MALAT1 alone, or co-transfected with pcDNA-MALAT1 and miR-382-3p mimic. As shown in Figure 6A, MALAT1 expression was elevated in IR HUVECs, and further increased when transfected with pcDNA-MALAT1, but decreased when co-transfected with pcDNA-MALAT1 and miR-382-3p mimic ($p < 0.05$). Moreover, the miR-382-3p expression determined by qRT-PCR was reduced in IR HUVECs, and further reduced when transfected with pcDNA-MALAT1, but markedly increased upon co-transfection with both pcDNA-MALAT1 and miR-382-3p mimic ($p < 0.05$) (Figure 6B). Next, western blot analysis revealed that resis-

tin expression ascended in IR HUVECs and further increased when transfected with pcDNA-MALAT1 but downregulated upon co-transfection with both pcDNA-MALAT1 and miR-382-3p mimic ($p < 0.05$) (Figure 6C). Glucose uptake was reduced in IR HUVECs and further decreased after overexpressing MALAT1, which could be rescued by miR-382-3p mimic transfection ($p < 0.05$) (Figure 6D). Transwell assay revealed that migration of IR HUVECs was promoted and pcDNA-MALAT1 transfection resulted in an enhancement of cell migration, however, which was inhibited by co-transfection with pcDNA-MALAT1 and miR-382-3p mimic ($p < 0.05$) (Figure 6E). At last, ELISA displayed that NO level was reduced and Ang II level was increased in the IR HUVECs and the IR HUVECs overexpressing MALAT1, both of which were reversed by restoration of miR-382-3p ($p < 0.05$) (Figure 6F). Taken together, these findings demonstrated
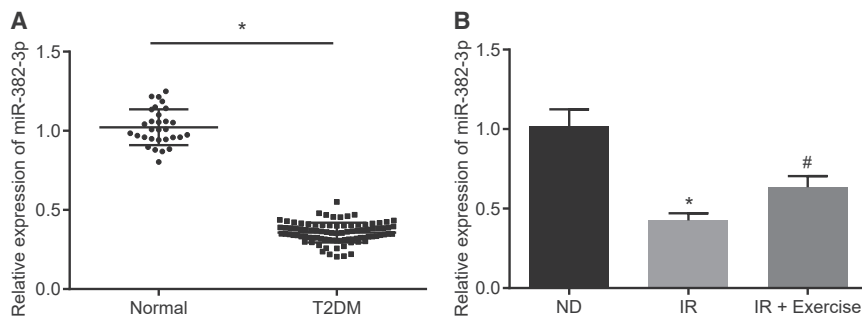
Figure 5. Exercise Relieves IR by Elevating miR-382-3p Expression

(A) miR-382-3p expression in healthy individuals (n = 30) and patients with T2DM (n = 90) determined using qRT-PCR; *p < 0.05, versus healthy individuals. (B) miR-382-3p expression in serum of IR mice or IR mice subjected to exercise detected using qRT-PCR; *p < 0.05, versus mice fed a normal diet; #p < 0.05, versus IR mice; measurement data were depicted as mean ± SD; comparisons between two groups were analyzed using unpaired t test; comparisons among multiple groups were analyzed by one-way ANOVA.

that MALAT1 could aggravate IR in HUVECs by impairing miR-382-3p-induced inhibition of resistin.

DISCUSSION

It is reported that about 90% of people diagnosed with diabetes suffer from T2DM.[21] T2DM is characterized by impaired insulin secretion and IR, the burgeoning incidence of which leads to a heavy burden on society.[1] In our previous study, we suggested that exercise might reduce IR by increasing miR-492 and reducing resistin.[22] However, more detailed mechanisms remain to be defined. The current study examined the suppressive role of exercise in IR in an attempt to elucidate the potential mechanisms associated with MALAT1, miR-382-3p, and resistin, which revealed that reduced MALAT1 expression produced by exercise could potentially reduce IR in T2DM by increasing miR-382-3p expression via synchronous inhibition of resistin.

Initially, the current study unveiled that MALAT1 was upregulated in T2MD and positively related to HOMA-IR and resistin. Consistently, MALAT1 has been found to be highly expressed in rats with diabetes.[14] Meanwhile, Hu et al. have also supported that MALAT1 expression was increased in kidney cortices of C57BL/6 mice with diabetic nephropathy induced by streptozocin (STZ).[23] Resistin in serum is correlated with HOMA-IR of diabetic subjects and has been implicated in obesity-associated T2DM.[24] Further, resistin is upregulated in obesity-mediated IR and affects the pathogenesis of obesity and IR.[18] The aforementioned were consistent with our findings that resistin was in a positive correlation with IR.

Moreover, the findings of dual-luciferase reporter and RIP assays confirmed that MATAT1 could competitively bind to miR-382-3p and consequently increase resistin expression. MALAT1 is demonstrated to act as a sponge of miRNA to regulate mRNA translation.[15] In addition, MALAT1 plays a role in Parkinson's disease by competitively binding to miR-124.[13] Also, another study has demonstrated that miRNAs participate in the development and progression of T2DM by regulating its target gene expression.[25] It has been revealed that dysregulated miRNA expression is correlated with T2DM.[26] Our team has previously demonstrated that miR-492 reverses HG-induced IR in HUVEC cells through directly inhibiting resistin expression.[27] These evidences suggest that knockdown of MALAT1

decreases resistin expression by impairing miR-382-3p-induced degradation of resistin, by which IR might be reduced.

Further, the findings of the present study confirmed that exercise could relieve IR via depletion of MALAT1 and resistin. The progression of T2DM is often affected by obesity, sedentary lifestyles, and lifestyle changes; for example, exercise could relieve T2DM.[3] Moreover, physical inactivity is one of the risk factors for IR and T2DM, and exercise serves as a therapeutic strategy for the prevention and treatment of IR in T2DM.[28] Also, as previously reported, MALAT1 depletion could obviously ameliorate diabetes.[14] MALAT1 is demonstrated to promote insulin sensitivity and act as a potential target for the treatment of diabetes.[29] MALAT1 ablation is demonstrated to increase insulin secretion and thus MALAT1 may act as a potential target for the treatment of diabetes.[29] Moreover, MALAT1 has been demonstrated to possess the ability to promote hepatic steatosis and IR.[30] Resistin, one of the members of a family of cysteine-rich secretory proteins, could induce IR and glucose intolerance,[31] which suggested that inhibition of resistin could relieve IR in T2DM. Endurance aerobic exercise or resistance training potentially contributes to the reduction in plasma resistin levels in the diabetic patients suffering from obesity and/or IR.[32] Similarly, exercise training protects against HFD-induced obesity by reducing oxidative stress and resistin and inhibiting vascular dysfunction.[33]

Another important finding of our study was that exercise could elevate miR-382-3p expression and consequently alleviate IR. Exercise-training-induced changes in miRNAs are demonstrated to be beneficial regulators in hypertension, T2DM, and obesity.[34] A study conducted by Melo et al. revealed that exercise training restores the cardiac miR-1 and miR-214 levels to improve ventricular function in myocardial infarction in rats.[35] Additionally, miR-712 restrains pro-inflammatory responses in macrophages to restore insulin-stimulated glucose uptake by myoblasts.[36] Furthermore, our experimental results revealed that silencing MALAT1 ameliorated IR by elevating miR-382-3p and disrupting resistin, which was evidenced by the promoted glucose uptake, elevated levels of NO and p-Akt/Akt, inhibited cell migration, and decreased levels of Ang II, TNF-α, IL-6, sICAM-1, sVCAM-1, ET-1, and p-IRS/ISR-1 in IR cell model. The inflammatory factors, including TNF-α and IL-6, are related to IR in T2DM.[37] In addition, several biological markers related to diabetic
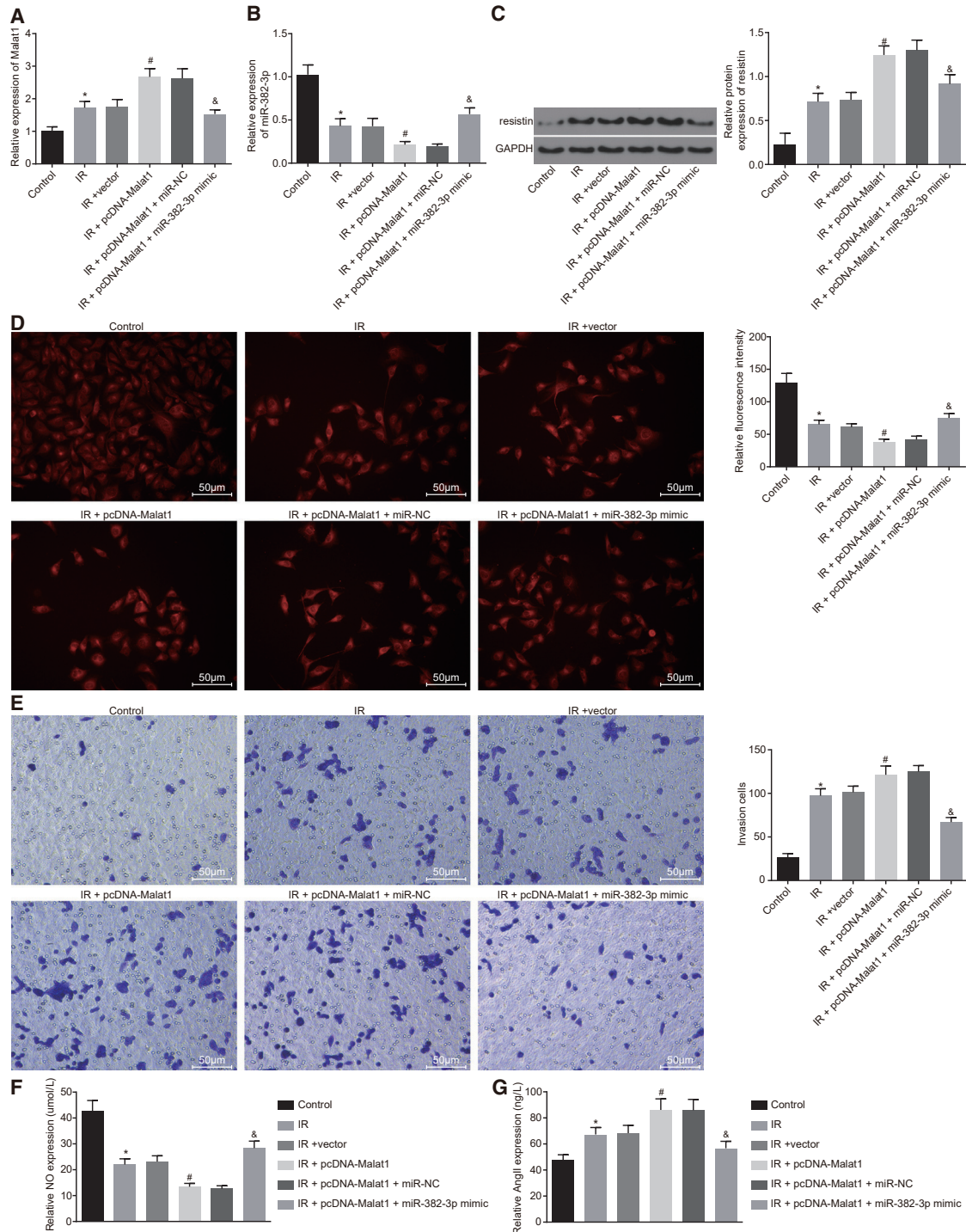
Figure 6. MALAT1/miR-382-3p/Resistin Axis Mediates IR in HUVECs

(A) Expression of MALAT1 determined using qRT-PCR. (B) Expression of miR-382-3p determined using qRT-PCR. (C) Western blot analysis of resistin protein. (D) Glucose uptake of HUVECs determined by glucose detection kit (200×). (E) HUVEC migration detected using Transwell assay (200×). (F) Serum level of NO in HUVECs measured using ELISA. (G) Serum level of Ang II in HUVECs measured using ELISA; $*p < 0.05$, versus cells incubated on DMEM supplemented with 5.5 mmol/L glucose; $\#p < 0.05$, versus cells incubated on the DMEM supplemented with 33 mmol/L glucose and transfected with vector plasmid; $\&p < 0.05$, versus cells incubated on the DMEM supplemented with 33 mmol/L glucose and co-transfected with pcDNA-MALAT1 and miR-NC plasmids; measurement data were depicted as mean ± SD; comparisons among multiple groups were analyzed by one-way ANOVA; the experiment was repeated three times.
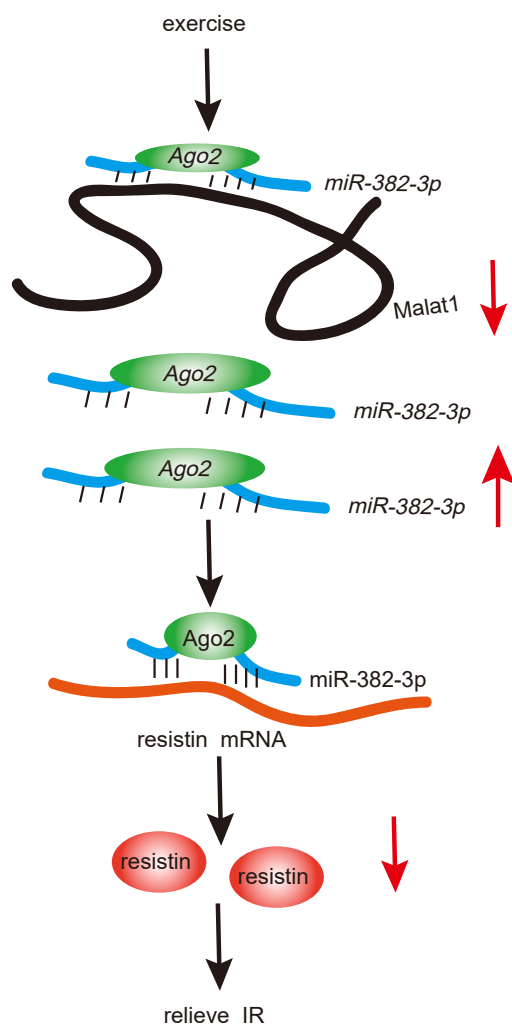
Figure 7. A Molecular Mechanism Map Depicting the Involvement of MALAT1/miR-382-3p/Resistin Axis in the Inhibitory Effect of Exercise on IR
Exercise could alleviate IR of HUVECs via reducing MALAT1 and resistin, and increasing miR-382-3p.

risk, such as sICAM-1 and sVCAM-1, are proposed as biomarkers of endothelial dysfunction.[38] Vascular inflammation and increased levels of sICAM-1 and sVCAM-1 were associated with IR.[39] Impairment in endothelium-dependent vasodilation is revealed by reduced NO level.[40] Furthermore, upregulation of exogenous NO is conducive to slow the development of diabetic vascular complications.[41] The abovementioned findings support that inhibition of MALAT1 overcomes IR, reduces the release of pro-inflammatory cytokines and adhesion molecules, and prevents endothelium dysfunction by increasing miR-382-3p and hindering resistin, which underlies the protective effect of exercise on IR.

In conclusion, the current study provides evidence suggesting that exercise can potentially downregulate MALAT1, which helps to alleviate IR in HUVECs in T2DM by elevating miR-382-3p and reducing

resistin (Figure 7). Thus, MALAT1/miR-382-3p/resistin axis facilitates a theoretical understanding of the mechanisms responsible for the protective role of exercise against IR in patients with T2DM. Further studies are warranted to elucidate the specific mechanism of MALAT1/miR-382-3p/resistin axis in IR in patients with T2DM based on the investigation about the potential effects of MALAT1 on HFD.

MATERIALS AND METHODS
Ethics Statement
The study was carried out with the approval of the Ethics Committee of Xiangya Hospital, Central South University. All the enrolled patients provided signed informed consents and all study procedures were compliant with the Declaration of Helsinki. All animal experiment procedures were performed in accordance with the protocols approved by the Animal Ethics Committee of Xiangya Hospital, Central South University.

Study Subjects
From June 2016 to June 2017, 90 patients with T2DM (56 males and 34 females aged 35–72 years with an average age of 55.48 ± 9.86 years) at Xiangya Hospital, Central South University were enrolled in the study. The included patients diagnosed with T2DM met the unified standards published by the International Diabetes Federation (IDF). The patients who were diagnosed with T2DM complicated with obesity or metabolism syndrome (MS) and those who had received insulin therapy were excluded. A total of 30 healthy volunteers (16 males and 14 females with an average age of 53.64 ± 9.24 years) without obesity or MS were recruited as the control group. Then the serum of patients was analyzed on an automatic biochemical analyzer (7160, Hitachi, Tokyo, Japan), after which the levels of FBG and blood lipid were measured, and serum insulin level was detected using an ELISA kit (PI608, Beyotime Biotechnology, Shanghai, China).

RNA Isolation and Quantitation
Total RNA from tissues was extracted using TRIzol kit (15596026, Invitrogen, Carlsbad, CA, USA). Next, the obtained RNA was reverse transcribed into cDNA according to the instructions of the PrimeScript RT reagent Kit (RR047A, Takara Bio Inc., Otsu, Shiga, Japan). qRT-PCR was then performed using a Fast SYBR Green PCR kit (Applied Biosystems, Carlsbad, CA, USA) on ABI PRISM 7300 RT-PCR system (Applied Biosystems, Carlsbad, CA, USA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and U6 were regarded as internal references for MALAT1 and miR-382-3p, respectively. Three replicates were set for each sample. The $2^{-\Delta\Delta Ct}$ method was adopted to analyze the expression of MALAT1 and miR-382-3p. The primer sequences are shown in Table 1.

IR Mouse Model Establishment
The mouse models of IR were constructed as previously described.[42] Briefly, specific pathogen-free (SPF) C57BL/6J male mice (aged 5–7 weeks) were purchased from Hunan SJA Laboratory Animal (Changsha, China). All mice were raised in a controlled SPF

Table 1. Primer Sequences for qRT-PCR

Gene	Primer Sequences
MALAT1	F 5′-AAAGTCCGCCATTTTGCCAC-3′
	R 5′-ACAACTCGCATCACCGGAAT-3′
miR-382-3p	F 5′-ATCCGTGAAGTTGTTCGTGG-3′
	R 5′-TATGGTTGTAGAGGACTCCTTGAC-3′
GAPDH	F 5′-CTCTGCTCCTCCTGTTCGAC-3′
	R 5′-GCGCCCAATACGACCAAATC-3′
U6	F 5′-GCTTCGGCAGCACATATACTAAAAT-3′
	R 5′-CGCTTCACGAATTTGCGTGTCAT-3′

MALAT1, metastasis-associated lung adenocarcinoma transcript 1; miR-382-3p, micro-RNA-382-3p; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; U6, U6 small nuclear RNA; F, forward; R, reverse.

environment with a temperature of $(21 \pm 3)°C$, a relative humidity of 55%–60%, 12 h day/night cycle, and free access to food and water. Among these mice, 10 mice were given granular standard feed, while 30 were fed with a 45% Kcal HFD (D12451; FBSH Bio-Pharmaceutical, Shanghai, China) to establish an IR model. The IR mice were randomly divided into 3 groups with 10 mice in each group respectively: HFD group (fed with HFD only), HFD + exercise group (fed with HFD and subjected to swimming), and HFD + sh-MALAT1 group (fed with HFD and injected with sh-MALAT1). After 12 weeks, the serum of mice was extracted and analyzed.

Biochemical Analysis
The concentration and contents of FBG, FINS, TC, TG, HDL-C, and LDL-C in the serum of mice in each group were analyzed using a Biochemical Analyzer (HITACHI 7020 Automatic Biochemical Analyzer, Tokyo, Japan).

ELISA
The serum samples were collected from mice in each group and centrifuged at 3,000 rpm/min for 5–10 min, after which the supernatant was collected. The levels of resistin, NO, IL-6, TNF-α, sICAM-1, and sVCAM-1 were measured in strict accordance with the instructions of their respective ELISA kits: resistin (JLC3371), NO (JLC0914), IL-6 (JLC1236), TNF-α (LC2062), sICAM-1 (JLC3133), and sVCAM-1 (JLC5564). All ELISA kits were manufactured by Shanghai Jingkang Bioengineering. (Shanghai, China).

Cell Culture and Transfection
HUVEC IR models induced by HG were constructed. In short, HUVECs were cultured with DMEM supplemented with 5.5 mmol/L glucose (normal group) or 33 mmol/L glucose (IR group). HUVECs of the IR group were transfected with the following plasmids respectively: sh-negative control (sh-NC), sh-MALAT1, blank vector pcDNA 3.1, MALAT1 overexpression vector (pcDNA-MALAT1), both pcDNA-MALAT1 and miR-NC, or both pcDNA-MALAT1 and miR-382-3p mimic. After incubation, the cells were cultured with serum-free DMEM (containing 5.5 mmol/L glucose) for 4 h, and then stimulated with insulin (5 mIU/L) for 10 min.

Western Blot Analysis
The total protein was extracted from cells using radio-immunopre-cipitation assay (RIPA) lysis buffer supplemented with protease inhibitor (Boster Biological Technology, Wuhan, Hubei, China). The protein concentration was detected using a bicinchoninic acid kit (Boster Biological Technology, Wuhan, Hubei, China). The extracted proteins were then separated by 10% sodium dodecyl sulfate-poly-acrylamide gel electrophoresis (SDS-PAGE), and transferred onto a polyvinylidene fluoride (PVDF) membrane. The membrane was blocked with 5% bovine serum albumin (BSA) for 2 h, and incubated overnight at 4°C with the following primary antibodies purchased from Abcam (Cambridge, MA, USA): mouse anti-resistin (ab136877, 1:500), mouse anti-ET-1 (ab2786, 1:1,000), rabbit anti-p-IRS (ab1194, 1:250), rabbit anti-IRS (ab52167, 1:1,000), and rabbit anti-p-Akt2 (ab38449, 1:1,000), and mouse anti-Akt2 (ab18785, 1:1,000). Subsequently, the membrane was incubated with the horse-radish peroxidase (HRP)-labeled goat anti-rabbit immunoglobulin G (IgG) (ab205718, 1:20,000, Abcam, Cambridge, UK) or rabbit anti-mouse IgG (ab6728, 1:5,000, Abcam, Cambridge, UK) at room temperature for 1 h. The enhanced chemiluminescence (ECL) (Millipore, Billerica, MA, USA) reagent was used to visualize the proteins on the membrane, followed by exposure to the X-ray film. The band intensities were quantified using ImageJ software. The ratio of the gray value of the target band to GAPDH was considered representative of the relative protein expression. The experiment was repeated three times.

Detection of Glucose Uptake
The glucose uptake was assessed according to the instructions of the Glucose Uptake Test kit (KA4085, Abnova, Taipei, China).

Transwell Assay
All cells were cultured in serum-free medium for 12 h and re-suspended with serum-free medium at a concentration of 1×10^5 cells/mL. The cell suspension (100 μL) was added to the apical Transwell chamber and 10% fetal bovine serum (FBS) was added to the basolateral chamber, followed by incubation at 37°C for 24 h. The cells in the apical chamber and those on the basement membrane were wiped off after incubation. The rest of the cells were fixed in 100% methanol for 10 min, stained with 1% toluidine blue (Sigma-Aldrich Chemical Company, St Louis, MO, USA), and then observed under an inverted optical microscope (Carl Zeiss, Jena, Germany). At last, the migrated cells were counted in five randomly selected visual fields. The experiment was repeated three times.

Dual-Luciferase Reporter Assay
A total of 2×10^5 cells were seeded into 6-well plates. After 48 h of transfection, the cells were collected. The luciferase activity of MATAT1, miR-382-3p, and resistin was detected according to the instructions of Dual-Luciferase Reporter Assay kit (D0010, Beijing solarbio Science & Technology, Beijing, China) using a GLoma × 20/20 Luminometer (E5311; Promega, Madison, WI, USA). The experiment was repeated three times.

RNA Binding Protein Immunoprecipitation Assay

The binding of MALAT1 and miR-382-3p with Ago2 was detected using an RNA binding protein immunoprecipitation kit (Millipore, Billerica, MA, USA) according to the manufacturer's instructions, with Ago2 antibody (1: 100, ab32381, Abcam Inc., Cambridge, MA, USA) or rabbit anti-human IgG (1: 100, ab109489, Abcam, Cambridge, MA, USA) as NC. After detachment with proteinase K, RNA was extracted and the expression of MALAT1 and miR-382-3p was determined using qRT-PCR.[43] Each experiment was repeated three times.

Statistical Analysis

Statistical analysis was performed using SPSS 21.0 software (IBM, Armonk, NY, USA), and the measurement data were expressed as mean ± SD. Data obeying normal distribution between two groups were compared using Student's t test. The skewed data were compared using Mann-Whitney U. test. Data among multiple groups were analyzed by one-way ANOVA, followed by a Tukey's multiple comparisons posttest. The correlation analysis was performed using Pearson's correlation coefficient. $p < 0.05$ was considered to be statistically significant.

AUTHOR CONTRIBUTIONS

S.-X.L., F.Z., and Y.C. designed the study. M.-R.X. and L.-J.J. were involved in data collection. S.-X.L. and K.-L.X. performed the statistical analysis and preparation of figures. S.-X.L., F.Z., K.-L.X., and Y.C. drafted the paper. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare no competing interests.

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