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miRNA-22 deletion limits white adipose expansion and activates brown fat to attenuate high-fat diet-induced fat mass accumulation

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ABSTRACT

Background: Obesity, characterized by excessive expansion of white adipose tissue (WAT), is associated with numerous metabolic complications. Conversely, brown adipose tissue (BAT) and beige fat are thermogenic tissues that protect mice against obesity and related metabolic disorders. We recently reported that deletion of miR-22 enhances energy expenditure and attenuates WAT expansion in response to a high-fat diet (HFD). However, the molecular mechanisms involved in these effects mediated by miR-22 loss are unclear.

Methods and Results: Here, we show that miR-22 expression is induced during white, beige, and brown adipocyte differentiation in vitro. Deletion of miR-22 reduced white adipocyte differentiation in vitro. Loss of miR-22 prevented HFD-induced expression of adipogenic/lipogenic markers and adipocyte hypertrophy in murine WAT. In addition, deletion of miR-22 protected mice against HFD-induced mitochondrial dysfunction in WAT and BAT. Loss of miR-22 induced WAT browning. Gain- and loss-of-function studies revealed that miR-22 did not affect brown adipogenesis in vitro. Interestingly, miR-22 KO mice fed a HFD displayed increased expression of genes involved in thermogenesis and adrenergic signaling in BAT when compared to WT mice fed the same diet.

Conclusions: Collectively, our findings suggest that loss of miR-22 attenuates fat accumulation in response to a HFD by reducing white adipocyte differentiation and increasing BAT activity, reinforcing miR-22 as a potential therapeutic target for obesity-related disorders.

Abbreviations: KO, knockout; WT, wild type; WAT, white adipose tissue; BAT, brown adipose tissue; EAT, epididymal adipose tissue; SAT, subcutaneous adipose tissue; HFD, high-fat diet; PIBA, primary immortalized brown adipocyte; PPARγ, peroxisome proliferator-activated receptor gamma; C/EBPα, CCAAT-enhancer-binding protein alpha; β3-AR, β3-adrenergic receptor; [^{18}F]-FDG, 2[^{18}F]-fluoro-2-deoxy-D-glucose; PET, positron emission tomography; SUV max, maximum standardized uptake value; Fabp4, fatty acid binding protein 4; Cfd, complement factor D; Adipoq, adiponectin; UCP1, uncoupling protein 1; Cidea, cell death-inducing DNA fragmentation factor, alpha subunit-like effector A; PRDM16, PR-domain containing 16; Tmem26, transmembrane protein 26; PGC1α, peroxisome proliferator activated receptor, gamma, coactivator 1 alpha; VDAC1, voltage-dependent anion-selective channel 1; ATP5A, ATP synthase lipid-binding protein; CREB, cAMP-response element binding protein; GLUT4, glucose transporter type 4; HSL, hormone sensitive lipase; ATGL, adipose triglyceride lipase; FAS, fatty acid synthase; Dio2, deiodonase-2; Gyk, glycerol kinase; Thra, thyroid hormone receptor alpha; Thrb, thyroid hormone receptor beta; Adrb1, beta-1 adrenergic receptor; Adrb3, beta-3 adrenergic receptor.

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1. Introduction

Chronic periods of positive energy balance result in weight gain and obesity, two highly prevalent conditions worldwide [1]. Obesity is characterized by excessive expansion of WAT and represents an important risk factor for several metabolic and cardiovascular disorders [2].

WAT expansion results from a combination of hypertrophy and hyperplasia, which involves proliferation and commitment of mesenchymal stem cells to preadipocytes followed by differentiation into mature adipocytes [3]. Diverse factors govern the expansion of adipose tissue depots, including C/EBP, that controls the early stages of differentiation, and PPARγ, which stimulates adipocyte maturation [3]. Despite intensive research efforts, the characterization of multiple mechanisms involved in control of WAT expansion are still unclear. In this regard, identifying molecular pathways that regulate white adipocyte function is critical for the development of novel and more effective therapies to treat obesity and its associated complications.

In contrast to white adipocytes, brown adipocytes exhibit high mitochondrial density and are specialized in energy dissipation through heat via UCP1. Several reports have demonstrated that BAT plays a key role in regulating energy balance and the thermogenic response [4,5]. In addition to brown adipocytes, certain stimuli induce the recruitment of thermogenic adipocytes expressing UCP1 within WAT, through a process referred to as browning. These adipocytes, known as "beige" or "brite" adipocytes, exhibit features similar to brown adipocytes such as multilocular lipid droplets and high mitochondria content. Whether beige adipocyte thermogenesis contributes to whole-body energy expenditure is still matter of debate [6].

Various stimuli lead to the recruitment of brown and beige adipocytes, such as cold exposure, pharmacological β3-AR agonist, and PPARγ activation [7]. These effects seem to be mediated by several transcription factors and coregulators including C/EBPβ, PGC1a, and PPARγ [8].

There is an inverse correlation between the thermogenic activity of BAT and body mass index and adiposity [9,10]. Increased BAT activation and WAT browning have been shown to stimulate whole-body energy expenditure, reduction of adiposity and protection against diet-induced metabolic dysfunction [4,11,12]. In this regard, a better understanding of the molecular signatures involved in the activation of brown/beige adipocytes becomes a promising strategy to develop better therapies to counteract the metabolic complications associated with obesity [5,7,13,14].

MiRNAs are small non-coding RNA molecules that function in RNA silencing and post-transcriptional regulation of gene expression; therefore, affecting myriad intracellular processes in health and disease [15]. Recent studies indicate that miRNAs play a key role in the control of white, brown, and beige adipocyte differentiation, as well as metabolism, and secretory and inflammatory responses [16–18]. We recently reported that miR-22 levels are elevated in WAT of obese mice and that loss of miR-22 attenuates fat mass gain in response to HFD by increasing energy expenditure [19]. Although the impact of miR-22 in obesity has been shown, the specific mechanisms by which miR-22 mediates these events remain to be elucidated.

In this study, we investigated the impact of miR-22 in white, brown, and beige adipocytes. Here, we report that miR-22 deletion reduces white adipogenesis but does not affect brown adipogenesis in vitro. Moreover, miR-22 deletion increased expression of genes involved in thermogenesis and adrenergic signaling in BAT of obese mice. Together, these findings reveal that miR-22 is a key regulator of white adipogenesis and brown fat function.

2. Materials and methods

2.1. Animal experimentation

All animal experimental protocols were approved by the Animal Care Committee of the Institute of Biomedical Sciences of the University of Sao Paulo (#66/2016/CEUA) and the IACUC (#15–08-2986R) of Boston Children's Hospital. The systemic miR-22 KO (for miR-22-3p and miR-22-5p) and WT mice used in this study were characterized previously [20] and were generated from heterozygous intercrosses. Five-week-old male miR-22 KO and WT mice were randomized and treated with a normal chow diet (10% kcal fat) and HFD (60% kcal fat) for 12 or 16 weeks (Teklad, Envigo, Madison, WI). We also treated male C57Bl/6 mice with the same diets for 8 weeks. Mice were housed at 22 ± 1 °C in a 12 h light-dark cycle and had water and food ad libitum. Body weight gain was monitored weekly. After diet treatment, mice were euthanized using carbon dioxide chamber and tissues were collected to mitochondrial analysis or frozen for further experiments. A fragment of these tissues was used for histological analysis. Detailed methods of in silico analysis, qPCR, western blotting, histological analyzes, triglyceride analysis, mitochondrial function, cytochrome *c* oxidase activity, cold exposure and rectal thermometry, and PET imaging are provided in the "Supplementary Material".

2.2. Cell culture

For white adipocytes, 3 T3-L1 cells were maintained in DMEM containing 4.5 g/l glucose supplemented with 10% FBS and 5 mM L-glutamine. The 3T3-L1 cells were induced to differentiate into white adipocytes in medium supplemented with 0.125 mM indomethacin, 1 μM dexamethasone, 0.5 mM isobutylmethylxanthine and 1 μM insulin for 7 days. CRISPR/Cas9 genome editing was used to knockout miR-22-3p and miR-22-5p in 3T3-L1 cells. Oil Red O staining was used to evaluate the intracellular lipid droplets. These methods are detailed in the "Supplementary Material".

Brown and subcutaneous adipocytes were generated in-house. Brown adipocytes were isolated from BAT of newborn C57/Bl6 mice and subcutaneous adipocytes from SAT of one-month old C57/Bl6 mice by collagenase digestion using standard protocol [21–23]. Preadipocytes were immortalized by infection with the retroviral vector pBabe, encoding SV40T antigen and selected with puromycin (1 μg/ml). Preadipocytes were grown to confluence in DMEM supplemented with 10% FBS and 5 mM L-glutamine. For PIBA differentiation, the medium was supplemented with 0.125 mM indomethacin, 1 μM dexamethasone, 0.5 mM isobutylmethylxanthine, 20 nM insulin, 1 nM T3 and 0.5 μM rosiglitazone for 6 days. Subcutaneous preadipocytes were induced to differentiate in brown/beige adipocytes with medium supplemented with 0.125 mM indomethacin, 1 μM dexamethasone, 0.5 mM isobutylmethylxanthine, 100 nM insulin, 1 nM T3 and 0.5 μM rosiglitazone for 4 days. Then, adipocytes were treated with 10 μM isoproterenol for 6 h [24].

For transient transfection assays, preadipocytes were seeded at 100% confluency. Then, cells were transfected with a miR-22-3p inhibitor (100 nM), negative control inhibitor (100 nM), negative control mimic (100 nM) or miR-22-3p mimic (100 nM) (Table S1) using Lipofectamine RNAimax (Life Technologies), according to the manufacturer's protocols. One day after transient transfection, the differentiation was induced as previously described and the transfection was repeated every 48 h. At the end of the differentiation process, transfection efficiency was determined by qPCR.

2.3. Statistical analyses

Shapiro-Wilk test was used to evaluate normality of the data prior the comparisons. Data were expressed as mean ± standard error. Statistical significance was calculated using Student's *t*-test, one-way ANOVA or two-way ANOVA followed by Tukey post hoc test using GraphPad® Prism software. Values of $p < 0.05$ was considered statistically significant.

3. Results

3.1. MiR-22 levels are increased in WAT of obese mice but not in skeletal muscle

Recently, we reported that miR-22 is abundant in WAT, liver, and heart of obese mice [19]. We thus decided to investigate whether HFD could affect miR-22 expression in other tissues. To address this question, C57Bl/6 male mice were fed a control or HFD for 8 weeks and miR-22 expression levels were evaluated in different tissues. Analysis of qPCR showed that miR-22-3p levels were markedly higher in skeletal muscle of C57Bl/6 mice compared to those observed in fat depots (Fig. S1A). However, HFD did not affect miR-22-3p levels in skeletal muscle. In line with our previous findings [19], mice fed a HFD exhibited increased miR-22-3p expression in EAT (Fig. S1B). Similarly, miR-22-3p expression was higher in SAT in response to a HFD (Fig. S1C).

3.2. Deletion of miR-22 reduces white adipocyte differentiation

Next, we performed in silico analysis using TargetScan to identify the potential biological targets of mouse miR-22-3p. The target prediction analysis of miR-22-3p revealed 445 genes (Table S2), which were used for enrichment analysis. The 10 most enriched pathways are presented on Fig. 1A. The miR-22 target genes annotated in each of the 10 most enriched pathways from the in silico analysis are shown on Table S3. Interestingly, one of the most enriched biological processes identified was adipogenesis.

To determine the role of miR-22 in white adipogenesis, we performed in vitro studies. First, we evaluated the miR-22-3p expression during the course of white adipogenic induction in 3T3-L1 cells. Analysis of qPCR revealed that miR-22 levels were enhanced at days 5 and 7 during white adipocyte differentiation (Fig. 1B). The mRNA levels of *Pparg*, an adipogenic transcriptional factor, were increased at 3, 5, and 7 days during white adipocyte differentiation, which were accompanied by increased lipid accumulation, as evaluated by Oil Red O staining (Fig. 1B). These findings suggest that miR-22 is expressed in white adipocytes, and its increased expression is observed at later stages of white adipocyte differentiation.

To further characterize the role of miR-22 in white adipocyte differentiation, we performed gain- and loss-of-function studies. Analysis of qPCR revealed that the expression of miR-22-3p was markedly increased in 3T3-L1 cells transfected with a miR-22 mimic compared with their respective controls (Fig. 1C). Overexpression of miR-22 did not affect the lipid content during white adipocyte differentiation (Fig. 1C,G). Consistent with this finding, overexpression of miR-22 did not change the triglyceride content in white adipocytes (Fig. 1D). However, overexpression of miR-22 in 3T3-L1 cells increased the mRNA levels of adipogenic markers such as *Pparg*, *Fabp4*, *Cfd,* and *Adipoq*, when compared with those found in control cells (Fig. 1E).

We therefore examined whether the loss of miR-22 could affect white adipocyte differentiation in vitro. For this, we used a CRISPR/Cas9 editing strategy to delete miR-22-3p and miR-22-5p in 3T3-L1 cells (Fig. S2). Deletion of miR-22 decreased miR-22 expression both in 3T3-L1 preadipocytes and adipocytes (Fig. 1F). Interestingly, miR-22 deletion reduced differentiation of 3T3-L1 cells into white adipocytes as evaluated by decreased lipid accumulation (Fig. 1F-G) and triglyceride content (Fig. 1D). In agreement with this finding, deletion of miR-22 reduced the mRNA levels of *Pparg*, *Cebpa*, *Fabp4*, *Cfd,* and *Adipoq* (Fig. 1H), suggesting that removal of miR-22 impairs white adipocyte differentiation.

3.3. Deletion of miR-22 reduces adipogenic/lipogenic gene expression in WAT of obese mice

To identify the underlying mechanisms by which miR-22 regulates WAT expansion in obesity, we fed WT and miR-22 KO mice with a control and HFD for 12 weeks. Both WT and miR-22 KO mice fed a HFD gained more body weight when compared with their respective controls fed a chow diet (Fig. S3A). As previously reported [19], loss of miR-22 did not affect HFD-induced body weight gain (Fig. S3A); however, attenuated HFD-induced EAT and SAT gain (Fig. S3B-C). Histological analysis of EAT (Fig. 2A, C) and SAT (Fig. 2B, D) demonstrated that adipocyte area was increased in WT mice fed a HFD. Whereas, miR-22 KO mice fed a HFD exhibited smaller adipocytes in EAT and SAT compared with those found in WT mice fed the same diet.

Triglyceride content was increased in EAT of WT mice fed a HFD (Fig. 2E). However, loss of miR-22 prevented the increase in triglyceride content in response to a HFD. qPCR analysis revealed that mRNA levels of *Cebpa*, *Fabp4* and *Cd36* were higher in EAT of obese WT mice compared with their respective controls (Fig. 2F). Similarly, mRNA levels of *Pparg* were increased in EAT of obese WT mice (Fig. S3D). In contrast, miR-22 deletion abrogated the increase in expression of adipogenic and lipogenic markers in EAT in response to a HFD. *Pparg* levels were higher in SAT of obese WT mice compared to their respective controls (Fig. 2G); however, loss of miR-22 prevented the increase in *Pparg* levels in response to a HFD. Together, these findings suggest that miR-22 is a key regulator of adipogenic/lipogenic markers in EAT in response to a HFD.

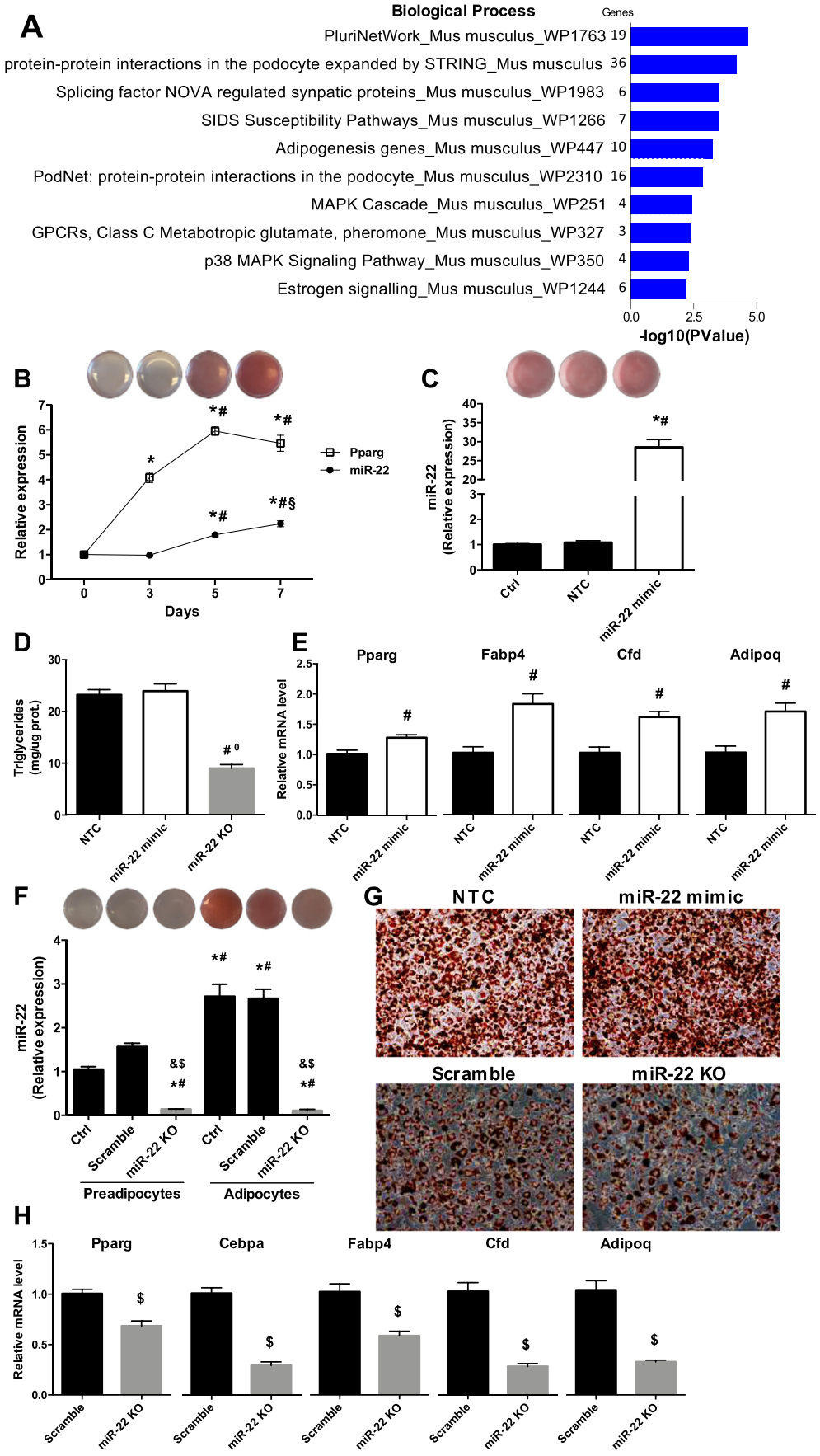
We therefore investigated whether loss of miR-22 affects mitochondrial metabolism by measuring oxygen consumption in isolated mitochondria from EAT. No differences were detected in mitochondrial oxidative phosphorylation from EAT between WT and miR-22 KO mice fed a control diet (Fig. 2H). However, isolated mitochondria from EAT of WT obese mice had reduced FCCP-induced maximal O_2 consumption when compared with their respective controls. Surprisingly, FCCP-induced maximal O_2 consumption was unchanged in isolated mitochondria from EAT of HFD-fed miR-22 KO mice in relation to their respective controls, suggesting that deletion of miR-22 protects against obesity-induced mitochondrial dysfunction.

In addition, the levels of *Pgc1a*, a mitochondrial biogenesis marker, were increased in SAT of miR-22 KO mice fed a control diet compared with WT mice fed the same diet (Fig. 2G). Moreover, miR-22 KO mice fed a HFD exhibited higher *Pgc1a* mRNA levels compared with WT obese mice (Fig. 2G). Also, mitochondrial cytochrome *c* oxidase activity was increased in SAT of miR-22 KO mice fed a control diet compared to WT mice fed the same diet (Fig. 2I).

Given that skeletal muscle is essential for energy homeostasis [25], we also evaluated mitochondrial oxygen consumption in skeletal muscle of the mice and found that neither a HFD nor deletion of miR-22 altered the oxygen consumption of mitochondria from skeletal muscle (Fig. S4). These findings suggest that loss of miR-22 in WAT rather than skeletal muscle may be the main source of the beneficial effects under a HFD.

3.4. Loss of miR-22 induces browning of WAT

Next, we investigated whether miR-22 regulates WAT browning using in vitro studies. Four days after differentiation induction, primary subcutaneous adipocytes were stimulated with isoproterenol to induce browning. qPCR analysis revealed that miR-22-3p and *Ucp1* levels were higher after browning induction (Fig. 3A-B). To investigate the role of miR-22 in the browning effect, we performed gain- and loss-of-function studies. Primary subcutaneous adipocytes transfected with miR-22 inhibitor showed significant reduction of miR-22-3p levels compared to control cells, whereas cells transfected with miR-22 mimic exhibited increased miR-22-3p levels (Fig. 3C). The inhibition or overexpression of miR-22 did not seem to affect lipid accumulation after browning induction, as assessed by Oil Red O staining (Fig. 3C). Interestingly, we observed that the expression of brown fat markers, such as *Ucp1* and *Cidea*, were higher in cells transfected with miR-22 inhibitor and stimulated with isoproterenol compared to control (Fig. 3D-E). Overexpression of miR-22 did not affect *Ucp1* mRNA levels (Fig. 3D), but reduced *Cidea* mRNA levels (Fig. 3E) compared to control.


A
Biological Process
Genes
PluriNetWork_Mus musculus_WP1763 19
protein-protein interactions in the podocyte expanded by STRING_Mus musculus 36
Splicing factor NOVA regulated synpatic proteins_Mus musculus_WP1983 6
SIDS Susceptibility Pathways_Mus musculus_WP1266 7
Adipogenesis genes_Mus musculus_WP447 10
PodNet: protein-protein interactions in the podocyte_Mus musculus_WP2310 16
MAPK Cascade_Mus musculus_WP251 4
GPCRs, Class C Metabotropic glutamate, pheromone_Mus musculus_WP327 3
p38 MAPK Signaling Pathway_Mus musculus_WP350 4
Estrogen signalling_Mus musculus_WP1244 6
0.0 2.5 5.0
-log10(PValue)
B
Relative expression
Days
Pparg
miR-22
C
miR-22 (Relative expression)
Ctrl
NTC
miR-22 mimic
D
Triglycerides (mg/ug prot.)
NTC
miR-22 mimic
miR-22 KO
E
Pparg
Fabp4
Cfd
Adipoq
Relative mRNA level
F
miR-22 (Relative expression)
Ctrl
Scramble
miR-22 KO
Preadipocytes
Adipocytes
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NTC
miR-22 mimic
Scramble
miR-22 KO
H
Pparg
Cebpa
Fabp4
Cfd
Adipoq
Relative mRNA level
Scramble
miR-22 KO

Next, we investigated the effect of miR-22 deletion in browning of SAT and EAT. qPCR analysis showed that miR-22 KO mice fed a control diet exhibited elevated mRNA levels of brown and beige fat markers in SAT, such as *Ucp1*, *Cidea*, and *Tmem26* (Fig. 3F) compared to WT littermate controls. However, HFD-fed miR-22 KO mice displayed reduced mRNA levels of *Ucp1* and *Cidea* in SAT when compared with their respective controls. Similarly, miR-22 KO mice fed a control diet showed higher mRNA levels of *Prdm16*, *Cidea* and *Elovl6* in EAT compared to WT mice fed the same diet (Fig. 3G). In addition, HFD-fed miR-22 KO mice exhibited higher mRNA levels of *Prdm16*, *Cidea* and *Elovl6* in EAT compared to WT obese mice. Western blot analysis revealed that UCP1 protein levels were increased in SAT of miR-22 KO mice fed a chow diet (Fig. 3H). Conversely, miR-22 KO mice fed a HFD had reduced UCP1 protein levels in SAT compared to those found in miR-22 KO mice fed a chow diet. Together, these results suggest that loss of miR-22 induces browning of SAT and EAT.

3.5. miR-22 did not affect brown adipocyte differentiation in vitro

Given that loss of miR-22 reduced the white adipogenic program, we investigated whether miR-22 could affect brown adipocyte differentiation in vitro. We first evaluated the expression of miR-22 during the differentiation course of brown preadipocytes, using PIBA cells. PIBA cells exhibited a progressive increase of *Ucp1* mRNA levels during the differentiation process (Fig. 4A) and increased lipid accumulation, as assessed by Oil Red O staining. Interestingly, miR-22-3p expression was higher at day 6 and 8 during the course of brown adipocyte differentiation (Fig. 4A). These findings suggest that miR-22 is expressed during brown adipogenesis, and its expression is slightly increased at a later stage of brown adipocyte differentiation.

Next, we characterized the role of miR-22 in brown adipogenesis using gain- and loss-of-function strategies. Analysis of qPCR detected that miR-22 inhibition reduced miR-22-3p levels in brown adipocytes, while miR-22 mimic increased miR-22-3p expression (Fig. 4B). Interestingly, neither inhibition nor overexpression of miR-22 affected the lipid accumulation during brown adipocyte differentiation (Fig. 4C). Similarly, inhibition or overexpression of miR-22 did not affect the mRNA expression of *Ucp1*, *Cidea*, *Prdm16*, and *Pparg* in brown adipocytes (Fig. 4D). Overall, these results support the idea that although miR-22 is expressed during the course of brown adipocyte differentiation, miR-22 does not play a cell non-autonomous role in brown adipogenesis in vitro.

3.6. Deletion of miR-22 activates the brown fat program

Given that BAT plays an important role in regulating adiposity and energy expenditure, we asked whether a HFD could affect miR-22 expression in BAT. Analysis of qPCR revealed that miR-22-3p levels were increased in BAT of WT mice fed a HFD compared with their respective controls (Fig. 5A). No difference was observed in BAT weight between WT and miR-22 KO mice fed both diets (Fig. 5B). Histological analysis of BAT showed that WT mice fed a HFD presented unilocular adipocytes in comparison to those fed a chow diet (Fig. 5D). However, miR-22 KO mice fed a HFD exhibited multilocular adipocytes in BAT. Consistent with the histological analysis, BAT triglyceride content was increased in WT mice fed a HFD (Fig. 5C). However, loss of miR-22 prevented the increase in triglyceride content in response to a HFD.

We next investigated whether deletion of miR-22 affects the expression of factors involved in adipogenesis/lipogenesis (*Pparg, Srebp1 and Fasn)* and lipolysis (*Hsl* and *Atgl*) in BAT. The mRNA levels of *Pparg* and *Srebp1* were increased in BAT of obese WT mice compared to their respective controls (Fig. 5E). In contrast, deletion of miR-22 abrogated the increase in expression of adipogenic/lipogenic markers in BAT in response to a HFD. Similarly, mRNA and protein levels of FAS were reduced in BAT of miR-22 KO mice fed a HFD compared to their respective controls (Fig. 5E-G). The *Hsl* and *Atgl* mRNA levels were similar between the groups (Fig. 5E). However, phosphorylation levels of HSL were increased in WT mice fed a HFD compared to those fed a chow diet (Fig. 5F-G). Western blot analysis also revealed that miR-22 KO mice fed a control diet exhibited increased GLUT4 levels in BAT (Fig. 5F-G). Our results suggest that deletion of miR-22 did not stimulate lipolysis in BAT in response to a HFD. However, miR-22 KO obese mice have reduced lipogenesis in BAT.

We then explored whether deletion of miR-22 could affect the expression of thermogenic genes in BAT. Analysis of qPCR showed that miR-22 KO mice fed a control diet displayed increased *Ucp1* and *Prdm16* mRNA levels in BAT compared to WT mice fed a control diet (Fig. 5H). Furthermore, mRNA levels of *Ucp1*, *Prdm16*, and *Cidea* were higher in BAT of miR-22 KO mice fed a HFD compared to WT mice fed the same diet (Fig. 5H). WT obese mice showed increased mRNA levels of *Tmem26* in BAT; however, deletion of miR-22 mitigated the increase in *Tmem26* expression in response to a HFD (Fig. 5H). These data suggest that miR-22 deletion activates *Ucp1* and *Prdm16* expression in BAT irrespective of the diet.

Immunofluorescence analysis of BAT demonstrated that WT obese mice exhibited a marked reduction in UCP1 levels compared with chow diet-fed WT mice (Fig. 5I). However, miR-22 deletion prevented the HFD-induced reduction in UCP1 levels in BAT. In line with this finding, western blot analysis revealed that UCP1 protein levels were increased in BAT of miR-22 KO mice fed a HFD compared to WT obese mice (Fig. 5F-G). Together, these findings suggest that deletion of miR-22 stimulates the thermogenic program in BAT and prevents HFD-induced reduction in UCP1 in BAT.

To investigate whether deletion of miR-22 could affect the thermogenic capacity of BAT, mice were treated with a β3-AR agonist (CL316,243) during the last week of dietary feeding, and then were exposed to acute cold exposure. Both WT and miR-22 KO mice fed either a control or HFD, when housed at room temperature, displayed similar rectal temperatures (Fig. 6A). On the other hand, HFD-fed WT mice exhibited lower rectal temperatures in response to cold exposure compared to their respective controls, suggesting that HFD impaired cold-induced thermogenesis. Interestingly, miR-22 KO mice fed a HFD did not show a reduction in rectal temperature upon cold exposure. These findings suggest that deletion of miR-22 prevents the HFD-induced reduction of thermogenesis.

We therefore examined whether miR-22 could influence BAT activation induced by a β3-AR agonist using [^{18}F]-FDG PET. The BAT SUV max was increased in HFD-fed miR-22 KO mice in response to acute β3-AR stimulation (Fig. 6B-C), suggesting that deletion of miR-22 stimulates glucose uptake in BAT of obese mice.

Fig. 1. Deletion of miR-22 reduces white adipogenesis in vitro. Enrichment of biological processes of the potential miR-22-3p target genes in mouse using Enrichr (A). Here we present the 10 most significant processes ($p < 0.05$). The negative log (−log10 (*p*-value)) was used. Oil Red O staining and expression of miR-22 and *Pparg* during the adipocyte differentiation (B) ($n = 4$ Pparg; $n = 8$ miR-22). Oil Red O staining and expression of miR-22 (C) (n = 8 ctrl; n = 8 NTC; $n = 6$ miR-22 mimic). Triglyceride content (D) ($n = 3$ NTC; n = 3 miR-22 mimic; n = 6 miR-22 KO), and expression of adipogenic genes, such as *Pparg, Cebpa, Fabp4, Cfd* and *Adipoq* (E) ($n = 7$ NTC; n = 7 miR-22 mimic). Expression of miR-22 (F) (Preadipocytes $n = 9$ ctrl; n = 6 scramble; n = 4 miR-22 KO and Adipocytes n = 9 ctrl; n = 9 scramble; n = 7 miR-22 KO). Adipocytes stained with Oil Red O (G). Expression of adipogenic genes (H) (n = 7 scramble; $n = 5$ miR-22 KO). 3T3L1 control cells (Ctrl), cells transfected with negative control (NTC, 20 nM), CRISPR/Cas9 scramble cells (scramble), cells transfected with miR-22 mimic (miR-22 mimic, 20 nM) or knockout cells for miR-22 (miR-22 KO). The analyses were performed in preadipocytes or after 7 days of differentiation. Data expressed as mean ± standard error; $p < 0.05$ (B) *vs 0 day, #vs 3 days, §vs 5 days; (C-E) *vs ctrl, #vs NTC, ^{0}vs miR-22 mimic; (F—H) *vs ctrl and #vs scramble preadipocytes, &vs ctrl and $vs scramble adipocytes. The groups were compared by (B—D, F) one-way ANOVA and (E, H) *t*-test (two-tailed). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

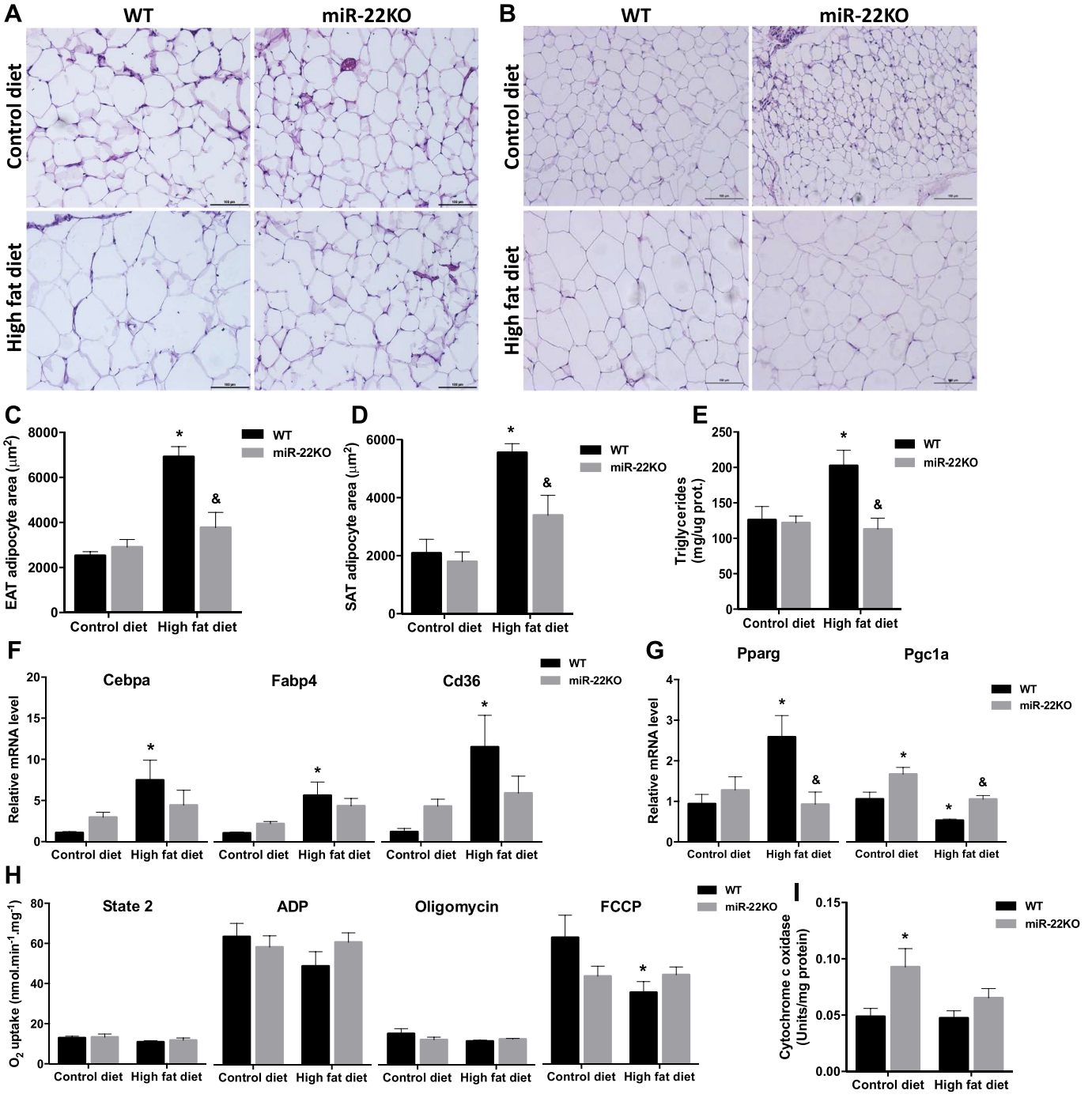

Fig. 2. Deletion of miR-22 reduces white adipogenesis in obese mice. Histology of EAT (A) and SAT (B) using hematoxylin and eosin; Adipocyte area quantification of the EAT (C) (n = 4 WTCD; n = 3 KOCD; n = 3 WTHFD; n = 3 KOHFD) and SAT (D) (n = 4 WTCD; n = 4 KOCD; n = 3 WTHFD; n = 4 KOHFD); Triglyceride content in EAT (E) (n = 5 WTCD; n = 5 KOCD; n = 6 WTHFD; n = 5 KOHFD); Analysis of gene expression in EAT (F) (n = 6 WTCD; n = 5 KOCD; n = 6 WTHFD; n = 7 KOHFD) and SAT (G) (n = 5 WTCD; n = 5 KOCD; n = 6 WTHFD; n = 6 KOHFD); Mitochondrial oxygen consumption in EAT (H) (n = 5 WTCD; n = 6 KOCD; n = 7 WTHFD; n = 7 KOHFD) and Cytochrome c oxidase activity in SAT (I) (n = 5 WTCD; n = 5 KOCD; n = 4 WTHFD; n = 5 KOHFD) of WT and miR-22KO mice fed a control diet and high-fat diet for 12 weeks (EAT) or 16 weeks (SAT). Data expressed as mean ± standard error; $p < 0.05$ *vs WT control diet, &vs WT high-fat diet. The groups were compared by two-way ANOVA (C—I).

Previous studies reported that enhanced mitochondrial biogenesis is associated with BAT thermogenic activation [5,26]. Accordingly, we tested whether deletion of miR-22 could affect mitochondrial biogenesis and oxygen consumption in BAT. We found that mitochondria isolated from BAT of HFD-fed WT mice have reduced mitochondrial state 2, maximal, and UCP1-dependent respiration in comparison to that from WT mice fed a chow diet (Fig. 6D). However, mitochondria isolated from BAT of HFD-fed miR-22 KO mice did not exhibit an alteration in oxygen consumption in relation to their respective controls, suggesting that miR-22 deletion prevents the disruption of mitochondrial respiration in BAT caused by obesity. In addition, mRNA expression of *Pgc1a* was higher in BAT of miR-22 KO mice fed a HFD compared to WT mice fed the same diet (Fig. 6E), which further confirms that loss of miR-22 helps to maintain mitochondria biogenesis in BAT of HFD-fed mice. Moreover, we evaluated the expression of proteins of different mitochondrial compartments, such as VDAC1 (outer mitochondrial

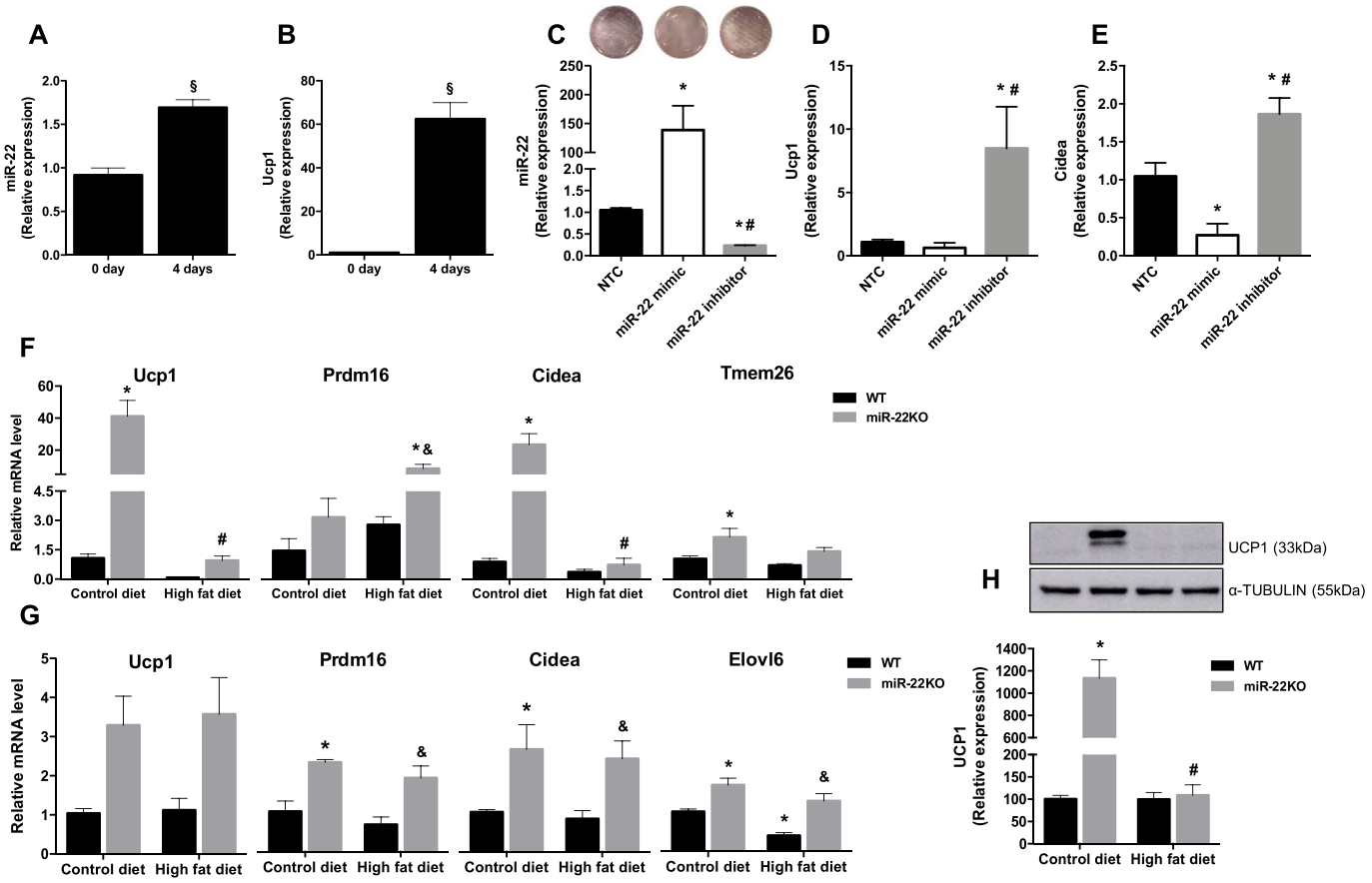

Fig. 3. Loss of miR-22 increases browning of WAT. Expression of miR-22 (A) (n = 5 0 day; n = 4 4 days) and *Ucp1* (B) (n = 3 0 day; n = 4 4 days) in primary subcutaneous preadipocytes (day 0) and after differentiation induction into beige adipocytes (4 days). Oil Red O staining and expression of miR-22 (C) (n = 7 NTC; n = 6 miR-22 mimic; n = 6 miR-22 inhibitor), *Ucp1* (D) (n = 6 NTC; n = 4 miR-22 mimic; n = 4 miR-22 inhibitor) and *Cidea* (E) (n = 4 NTC; n = 5 miR-22 mimic; n = 5 miR-22 inhibitor) in primary subcutaneous adipocytes transfected with negative control (NTC, 100 nM), miR-22 mimic (100 nM) or miR-22 inhibitor (100 nM) after 4 days of differentiation and stimulated with isoproterenol. Analysis of gene expression of browning markers in SAT (F) (n = 4 WTCD; n = 4 KOCD; n = 5 WTHFD; n = 5 KOHFD) and EAT (G) (n = 6 WTCD; n = 6 KOCD; n = 6 WTHFD; n = 7 KOHFD); and protein expression of UCP1 in SAT (H) (n = 6 WTCD; n = 5 KOCD; n = 7 WTHFD; n = 5 KOHFD) of WT and miR-22KO mice fed a control diet and high-fat diets for 12 weeks (EAT) or 16 weeks (SAT). Data expressed as mean ± standard error; $p < 0.05$ (A-B) §vs 0 day, *vs NTC; (C-E) *vs NTC, #vs miR-22 mimic; (F–H) *vs WT control diet, #vs KO control diet, &vs WT high-fat diet. The groups were compared by (A-B) *t*-test (two-tailed), (C-E) one-way ANOVA and (F–H) two-way ANOVA. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

membrane) and ATP5A (inner mitochondrial membrane). WT mice fed a HFD exhibited a marked reduction in VDAC1 and ATP5A levels compared to those fed a chow diet (Fig. 6F-G). On the other hand, deletion of miR-22 prevented HFD-induced reduction in VDAC1 and ATP5A levels in BAT. Together, these results suggest that deletion of miR-22 protects against obesity-induced mitochondrial dysfunction in BAT.

Next, we evaluated whether miR-22 regulates the adrenergic signaling in BAT. Analysis of qPCR showed that mRNA levels of adrenergic receptors (*Adrb1* and *Adrb3)*, *Gyk*, *Dio2*, and thyroid hormone receptors (*Thra* and *Thrb)* were increased in BAT of obese miR-22 KO mice (Fig. 6E). Inhibition of miR-22 also increased mRNA levels of *Thra* and *Thrb* in PIBA cells (Fig. S5). In addition, protein levels of TRα and phosphorylation levels of CREB were higher in BAT of miR-22 KO mice fed a HFD (Fig. 6F-G). Collectively, these findings suggest that deletion of miR-22 increases adrenergic signaling in BAT in response to a HFD.

In Fig. 7, we summarize the proposed mechanism of miR-22 function in balancing white adipogenesis, browning of WAT and BAT activation in obese mice. Our results show that loss of miR-22 reduced white adipogenic/lipogenic markers, as well as the size of adipocytes in WAT of HFD-fed mice. Furthermore, deletion of miR-22 induced WAT browning and increased the expression of thermogenic regulators such as *Pgc1a*, *Ucp1*, *Dio2*, *Thra*, *Thrb, Adrb1, Adrb3, and Gyk* in BAT in response to a HFD, leading to BAT activation.

4. Discussion

In recent years, several studies have investigated the biological mechanisms involved in WAT expansion, BAT activation, and browning of WAT to counteract the detrimental effects of obesity.

We recently showed that deletion of miR-22 attenuated HFD-induced adiposity, prevented dyslipidemia, and increased energy expenditure [19]. To gain insight into the mechanisms by which miR-22 controls adiposity and energy expenditure in obese mice, we used WT and miR-22 KO mice fed a control or HFD, as well as white, brown, and browning-induced subcutaneous adipocyte cultures.

Consistent with our previous work [19], we found that miR-22 levels are increased in EAT of WT obese mice. Although deletion of miR-22 attenuated fat mass accumulation in response to HFD compared to WT mice, body weight was not reduced in miR-22 KO mice after HFD. Recently, we found that miR-22 deletion in female mice attenuated body weight gain in response to an obesogenic diet [27]. Therefore, it would be interesting to investigate whether deletion of miR-22 in male mice may affect body weight gain after long-term exposition to HFD. Moreover, it would be interesting to examinate whether miR-22 deletion may increase loss of fat in feces or induce ectopic fat accumulation.

Histological analysis of EAT revealed that adipocyte area is increased in WT mice fed a HFD. However, miR-22 KO mice fed a HFD display reduced adipocyte area and triglyceride content in EAT compared with

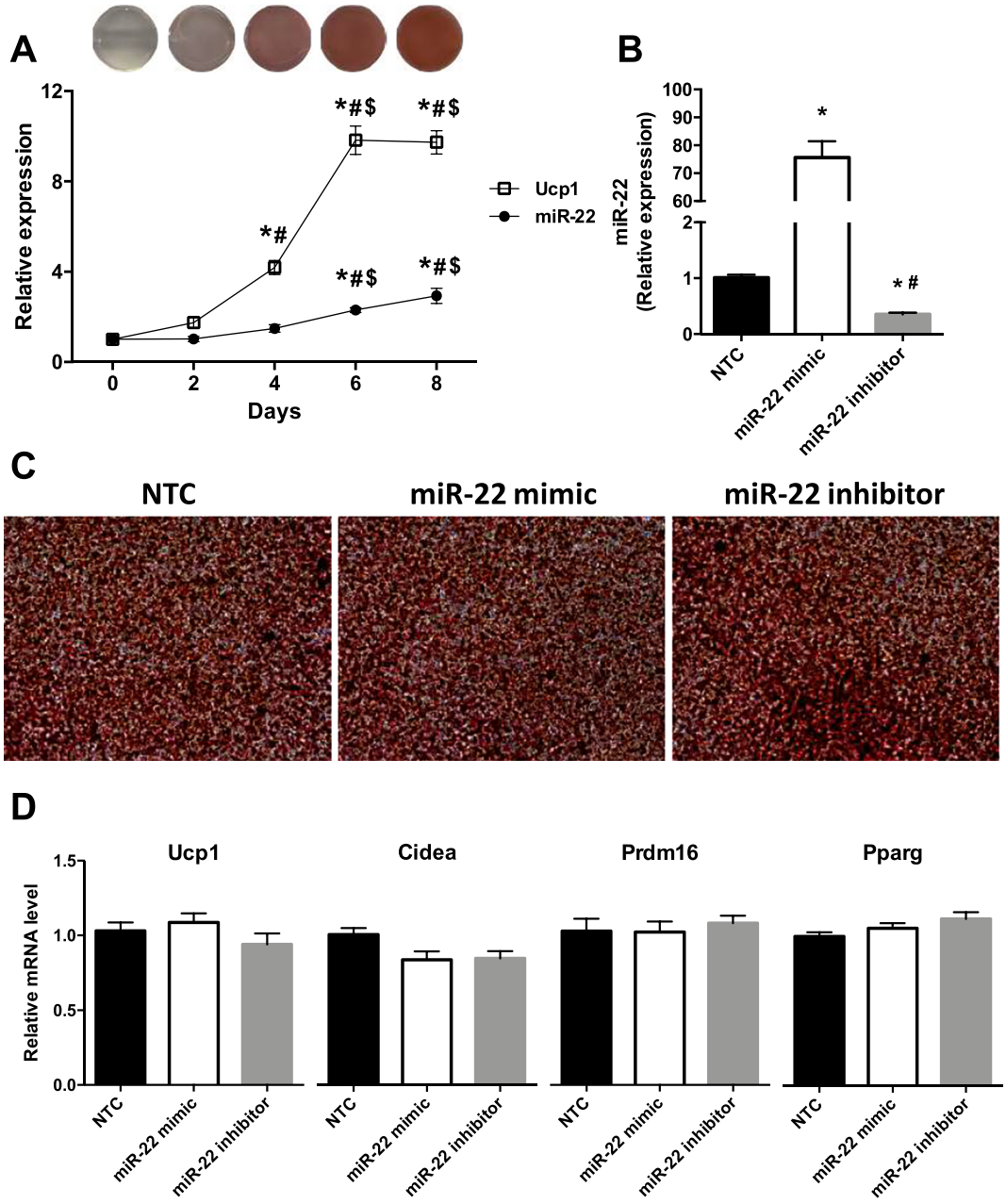

Fig. 4. miR-22 does not affect brown adipocyte differentiation in vitro. Expression of miR-22 and *Ucp1* during brown adipocyte differentiation (n = 6) and images of PIBA cells stained with Oil Red O were presented above the graph (A). Expression of miR-22 (B) (n = 9 NTC; n = 8 miR-22 mimic; n = 8 miR-22 inhibitor); Adipocytes stained with Oil Red O after 6 days of differentiation (C); Expression of *Ucp1*, *Cidea*, *Prdm16* and *Pparg* (D) (n = 9 NTC; n = 8 miR-22 mimic; n = 8 miR-22 inhibitor) in PIBA cells transfected with negative control (NTC, 100 nM), miR-22 mimic (100 nM) or miR-22 inhibitor (100 nM) after 6 days of differentiation. Data expressed as mean ± standard error; $p < 0.05$ (A) *vs 0 day, #vs 2 days, $vs 4 days; (B) *vs NTC, #vs miR-22 mimic. The groups were compared by one-way ANOVA. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

WT obese mice. Increased adipocyte diameter has been associated with an exacerbated pro-inflammatory state of WAT, contributing to obesity-related complications [28]. We have previously shown that miR-22 deletion prevented the increased gene expression of pro-inflammatory markers in EAT in response to HFD [19]. Moreover, we reported that loss of miR-22 reduces the expression of genes involved in adipogenesis in EAT [19]. Expanding upon these results, we observed that miR-22 deletion prevented the increase in expression of several other adipogenic/lipogenic markers in EAT of obese mice, like *Cebpa*, *Fabp4,* and *Cd36*.

To further support the involvement of miR-22 as a key factor of white adipocyte differentiation, we performed gain- and loss-of-function studies. Our results revealed that miR-22 expression levels are increased at day 5 and day 7 post-induction of differentiation, while *Pparg* expression levels are increased at day 3. In addition, miR-22 overexpression in 3T3-L1 cells increased the expression of adipogenic/lipogenic markers. These results contrast with a previous study, which demonstrated that miR-22 expression is reduced during adipogenic differentiation of human adipose tissue-derived mesenchymal stem cells and that overexpression of miR-22 reduced the expression of adipogenic factors and lipid accumulation [29]. Although the exact reasons for these discrepancies regarding the effect of forced miR-22 expression in white adipogenesis are unclear, differences in the cell type used (human vs murine), the purity of preadipocytes, the miR-22 overexpression strategy, and the transfection efficiency may explain these divergent results.

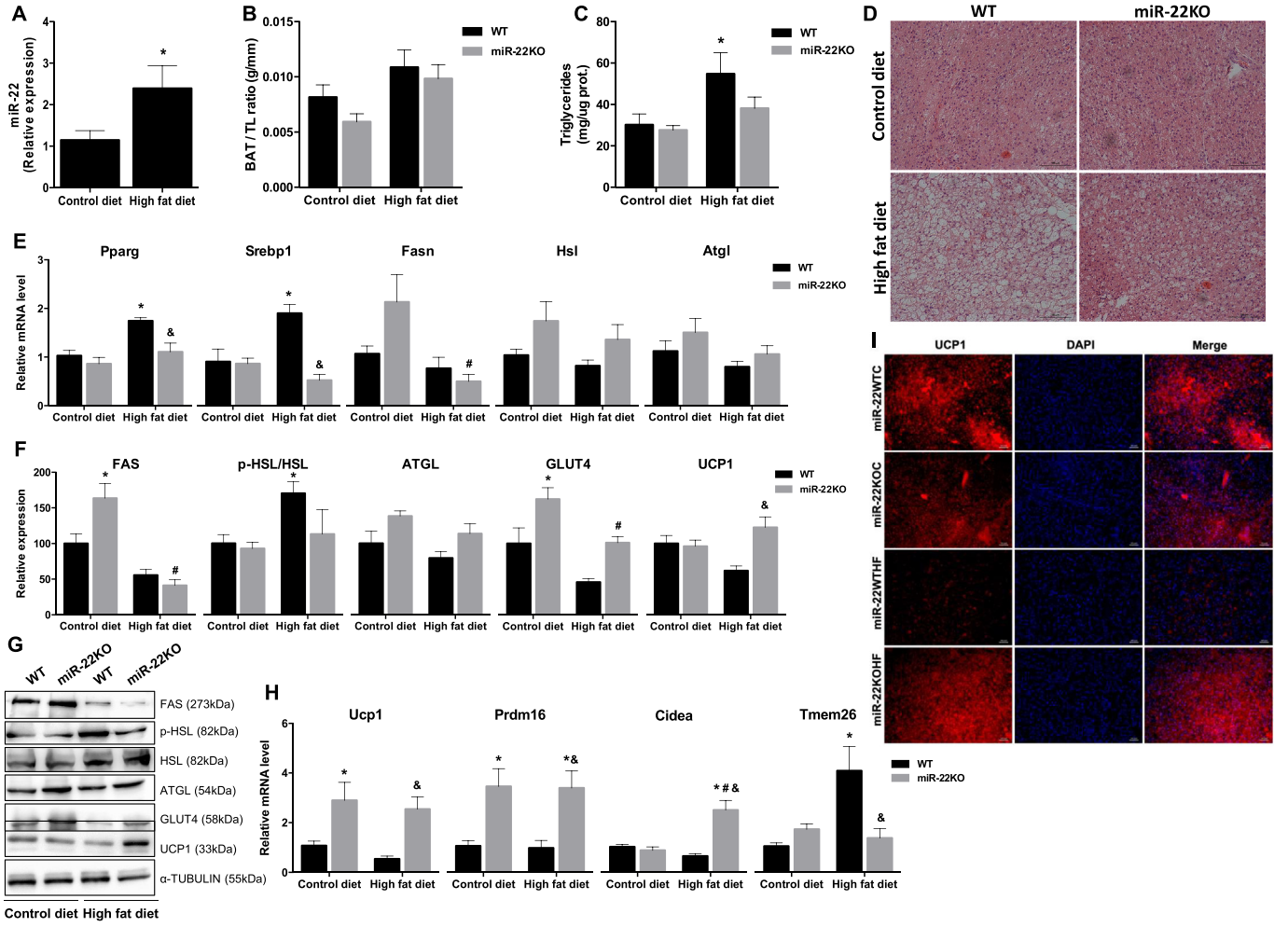

Fig. 5. Deletion of miR-22 activates the brown fat program in mice. Expression of miR-22 in brown adipose tissue (BAT) of WT mice (A) (n = 7); BAT weight normalized by tibia length (BAT/TL) (B) (*n* = 12 WTCD; n = 12 KOCD; *n* = 16 WTHFD; *n* = 15 KOHFD); Triglyceride content in BAT (C) (n = 5 WTCD; n = 6 KOCD; n = 4 WTHFD; n = 5 KOHFD); Histology of the BAT (D); Gene expression of *Pparg, Srebp1, Fasn, Hsl and Atgl* in BAT (E) (n = 6 WTCD; n = 6 KOCD; n = 7 WTHFD; n = 7 KOHFD); Protein levels (F) and western blotting representative images (G) of FAS, p-HSL, HSL, ATGL, GLUT4 and UCP1 (n = 6 WTCD; n = 6 KOCD; n = 5 WTHFD; n = 5 KOHFD); Gene expression of *Ucp1, Prdm16, Cidea* and *Tmem26* in BAT (H) (n = 7 WTCD; n = 6 KOCD; n = 6 WTHFD; n = 6 KOHFD); UCP1 immunofluorescence (I) in BAT of WT and miR-22KO mice fed a control diet and high-fat diet for 16 weeks. Data expressed as mean ± standard error; $p < 0.05$ *vs WT control diet, #vs miR-22KO control diet, &vs WT high-fat diet. The groups were compared by (A) *t*-test (two-tailed) and (B–H) two-way ANOVA. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

In this study, we used CRISPR/Cas9 miR-22 deletion on white adipogenesis because miR-22 inhibitor was not able to reduce miR-22-3p levels during 3T3L1 adipocyte differentiation. We found that deletion of miR-22 in 3T3-L1 cells reduces the expression of adipogenic/lipogenic markers and lipid accumulation upon induction of differentiation, indicating that loss of miR-22 reduces white adipocyte differentiation. Together, our findings suggest that miR-22 is a key regulator of the white adipogenic program, since deletion of miR-22 reduced the expression of adipogenic/lipogenic markers both in EAT in response to a HFD, as well as in white adipocyte in vitro. Based on these findings, we hypothesize that the reduced EAT fat pad and adipocyte area in miR-22 lacking mice fed a HFD may be mediated by reduced expression of adipogenic/lipogenic markers as result of miR-22 deletion.

In the present study, we have not evaluated the expression of miR-22 predicted target genes involved in adipogenesis. Previous studies showed that Klf7 and Wnt, which are predicted targets of miR-22, inhibit preadipocyte differentiation into adipocytes [30,31]. In addition, Sp1, another predicted target of miR-22, act repressively on the C/EBPα promoter prior adipocyte differentiation induction [32]. Moreover, Sp1 interacts with PPARγ to induce transcription of target genes [33].

Several studies have demonstrated that mitochondrial dysfunction in adipose tissue plays a key role in the pathogenesis of obesity-induced complications [34–38]. Our results reveal that HFD reduces oxygen consumption in mitochondria isolated from EAT and BAT of WT mice. However, miR-22 lacking mice fed a HFD did not display such reduction in oxygen consumption in EAT and BAT, suggesting that deletion of miR-22 protects against obesity-induced mitochondrial dysfunction.

Activation of thermogenic adipocytes can reduce adiposity and improve obesity-related metabolic complications in rodent models, suggesting that stimulation of WAT browning and/or activation of BAT might be a novel strategy to combat diet-associated metabolic disorders [13,39,40]. Most studies that have investigated beige adipocytes focused on subcutaneous deposition, which is highly susceptible to browning [41]. Our results reveal that miR-22 KO mice fed a HFD have a lower SAT weight and adipocyte area compared to WT obese mice. Although loss of miR-22 had induced WAT browning under a chow diet, UCP1 levels were reduced in SAT when mice were fed a HFD. In vitro experiments using subcutaneous preadipocytes revealed that *Ucp1* expression was strongly induced after stimulation with isoproterenol, while miR-22 expression was slightly increased. Our results in 3T3-L1 showed

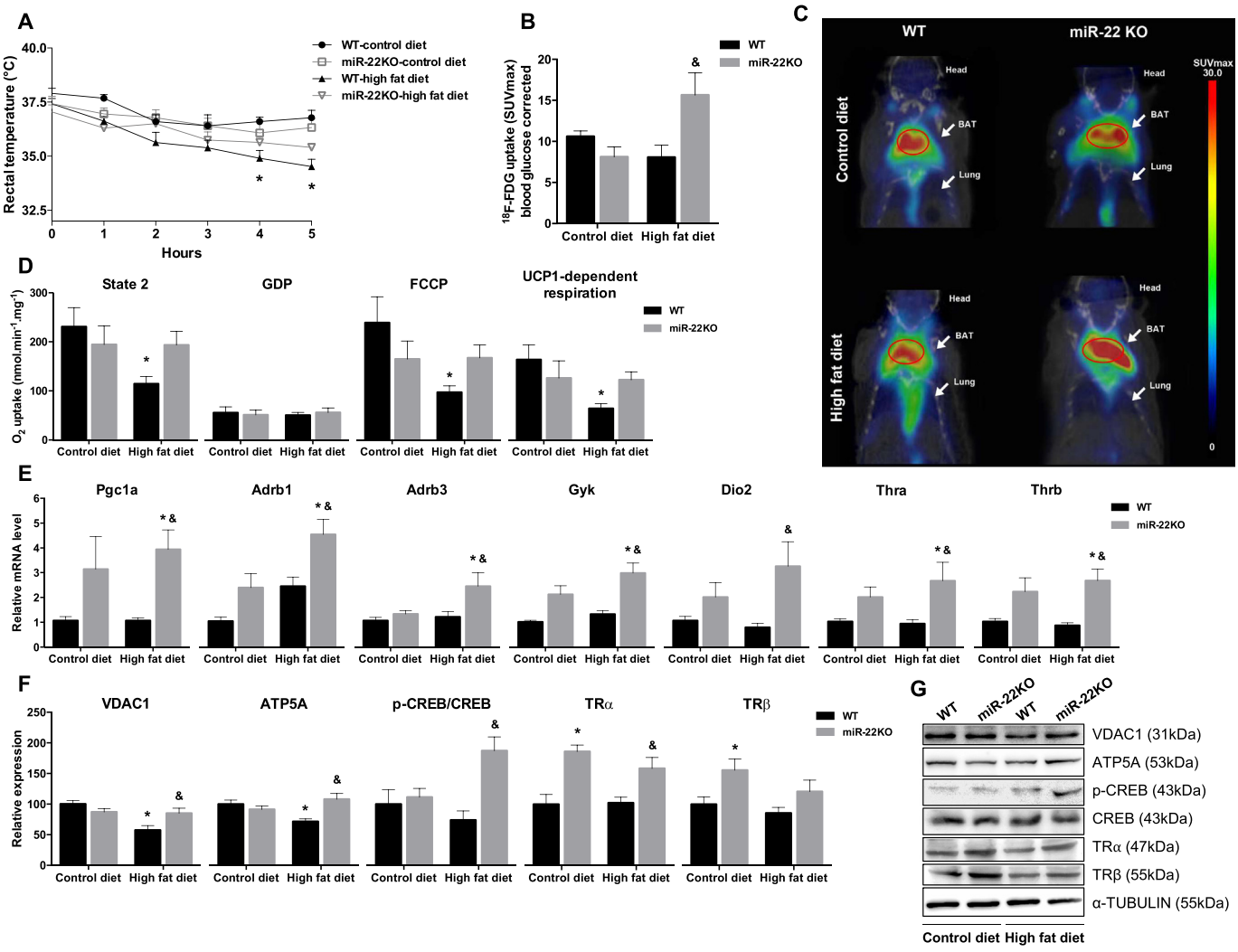

Fig. 6. Loss of miR-22 increases BAT activation in obese mice. Rectal temperature (A) (n = 4 WTCD; n = 4 KOCD; n = 7 WTHFD; n = 5 KOHFD); [^{18}F]-FDG uptake (SUVmax) (B) (n = 4 WTCD; n = 4 KOCD; n = 5 WTHFD; n = 6 KOHFD) and representative [^{18}F]-FDG PET-CT images (C); Mitochondrial oxygen consumption of BAT (D) (n = 6 WTCD; n = 6 KOCD; n = 6 WTHFD; n = 4 KOHFD); Gene expression of *Pgc1a, Adrb1, Adrb3, Gyk, Dio2, Thra,* and *Thrb* in BAT (E) (n = 5 WTCD; n = 8 KOCD; n = 6 WTHFD; n = 6 KOHFD); Protein levels of VDAC1, ATP5A, p-CREB/CREB, TRα and TRβ in BAT (F-G) of WT and miR-22KO mice fed a control diet and high-fat diet for 16 weeks (n = 5 WTCD; n = 6 KOCD; n = 8 WTHFD; n = 6 KOHFD). Data expressed as mean ± standard error; $p < 0.05$ *vs WT control diet, &vs WT high-fat diet. The groups were compared by two-way ANOVA.

that miR-22 expression was slightly increased at later stages of white adipocyte differentiation. Based on these findings, we believe that increased miR-22 expression in subcutaneous adipocytes after stimulation with isoproterenol in vitro may be due to the differentiation process. Interestingly, inhibition of miR-22 increased the expression of *Ucp1* and *Cidea* in subcutaneous adipocytes after stimulation with isoproterenol. Moreover, miR-22 KO mice fed a control diet displayed increased mRNA levels of *Ucp1*, *Cidea* and *Tmem26* in SAT, suggesting that miR-22 deletion induces SAT browning. The exact mechanisms involved in these events are unknow. We hypothesize that increased expression of miR-22 predicted target genes, such as *Pgc1a* and *Elovl6*, may induce WAT browning of miR-22 KO mice. However, future studies are required to elucidate this hypothesis. In addition, further investigations are needed to determine whether browning of WAT may be, at least in part, contributing to the beneficial effects promoted by miR-22 deletion in obese mice.

Our findings revealed that miR-22 itself does not affect brown adipogenesis, since neither forced expression nor inhibition of miR-22-3p affected brown adipocyte differentiation in vitro. Despite inhibition rate with CRISPR/Cas9 system in 3T3-L1 cells is higher than with miR-22-3p inhibitor in subcutaneous or brown adipocytes, it is important to note that neither inhibition or overexpression of miR-22 changed lipid accumulation in brown and subcutaneous adipocytes or affected expression of BAT markers in brown adipocytes, suggesting that this miRNA has few effects in these cells. Moreover, BAT from WT obese mice had unilocular adipocytes in comparison to those fed a chow diet. This finding is consistent with previous studies, which showed that BAT from obese mice has more unilocular adipocytes compared with lean mice [42–44]. However, miR-22 KO mice fed a HFD displayed multilocular adipocytes in BAT and reduced triglyceride content, suggesting that deletion of miR-22 prevents BAT whitening in response to a HFD.

miR-22 KO mice fed a HFD displayed increased expression of thermogenic genes (*Ucp1*, *Prdm16* and *Cidea*) and UCP1 protein levels in BAT, suggesting that deletion of miR-22 stimulates the thermogenic program in BAT. Previous studies showed that the major function of *Dio2* is to convert T4 to T3, which plays an important role enhancing adrenergic signaling and promoting the induction of both *Ucp1* [45] and *Pgc1a* in brown adipocytes [46]. *Pgc1a* activates several other transcription factors and acts as a central regulator of diverse pathways involved in mitochondrial biogenesis and thermogenesis [47]. It is also important to note that *Pgc1a*, which is the master regulator of UCP1-mediated

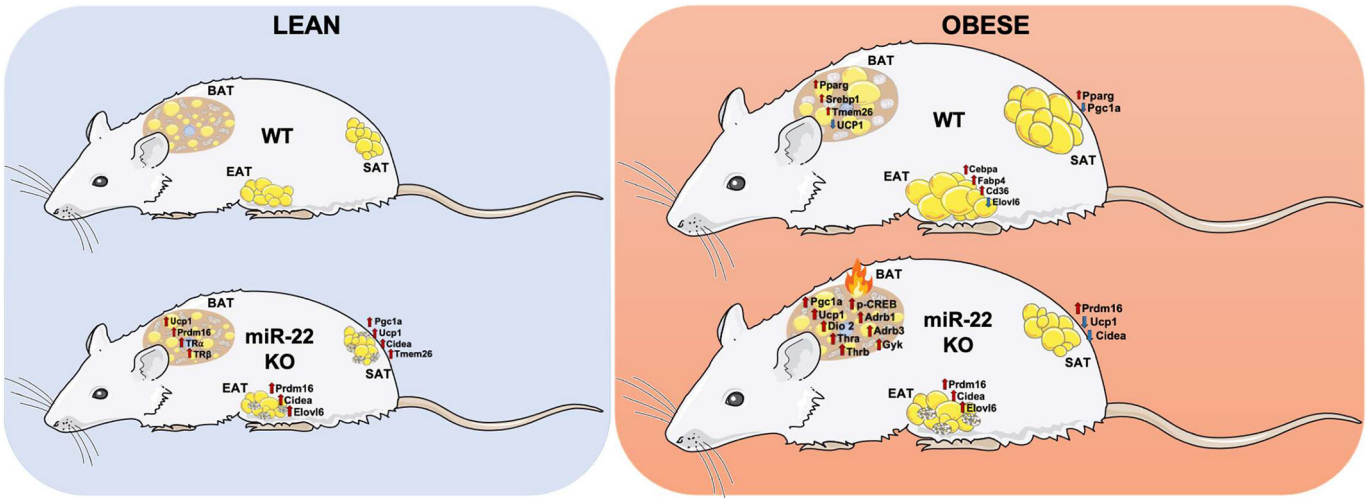

Fig. 7. Proposed mechanisms of action for miR-22 in white and brown fat. Loss of miR-22 reduced the adipocytes size in SAT and EAT of HFD-fed mice, as well as prevented the increase in adipogenic markers induced by HFD. Deletion of miR-22 increased the expression of brown/beige fat markers in WAT, such as *Ucp1*, *Cidea*, *Prdm16*, *Elovl6*, and *Tmem26*. Furthermore, deletion of miR-22 increased the expression of thermogenic genes such as *Pgc1a*, *Ucp1*, *Dio2*, *Thra*, *Thrb*, *Adrb1*, *Adrb3* and *Gyk* in BAT in response to HFD. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

thermogenesis in BAT, is a validated target of miR-22 [48,49] and its expression is increased in BAT of miR-22 KO mice fed a HFD. In addition, we found that *Thra* and *Thrb* expression was increased in BAT of miR-22 KO obese mice. Previous studies reported that *Thrb* affects adaptive thermogenesis by regulating UCP1, while *Thra* is required to maintain the adrenergic sensitivity in brown adipocytes [45,50]. Prediction analysis using TargetScan identified *Elovl6, Thra and Creb1* as predicted targets for miR-22-3p. Although future validation assays are required to verify whether miR-22-3p directly targets these genes, it is important to note that TRα protein levels and phosphorylated levels of CREB1 were increased in BAT of miR-22 KO obese mice.

BAT is the main site for cold-induced thermogenesis. Cold exposure and β3-AR agonists increase UCP1 expression and activate BAT and beige fat, influencing body temperature maintenance and whole-body energy expenditure [13,51]. WT obese mice had lower rectal temperature after cold exposure compared to chow diet-fed mice. This finding is consistent with previous studies, which demonstrated that HFD reduces the rectal temperature of mice, rats, and humans upon cold exposure [52–55]. However, miR-22 KO obese mice did not exhibit reduction in rectal temperature upon cold exposure, suggesting that loss of miR-22 prevents the reduction of thermogenesis in response to a HFD.

Activation of BAT may be quantified using [^{18}F]-FDG PET imaging [56]. Our results showed that HFD did not change [^{18}F]-FDG uptake in WT mice. In contrast, miR-22 KO mice fed a HFD exhibited increased tracer uptake than WT obese mice. Together, our findings provide strong evidence that deletion of miR-22 activates BAT function without changing brown adipogenesis. Some studies have shown two types of UCP1-positive adipocytes in BAT, the classical brown adipocytes and the brown-like adipocytes, which express Slc27a1, TMEM26, TBX1, CD40, and CD137 [57,58]. Although deletion of miR-22 did not increase *Tmem26* mRNA levels in BAT in response to a HFD, further studies are required to evaluate whether a possible transdifferentiation of unilocular adipocytes into multilocular adipocytes could be involved in the beneficial effects promoted by loss of miR-22 in obese mice.

BAT activity is inversely correlated with adiposity or body mass index [9,10]. Given that enhanced BAT function stimulates whole-body energy expenditure and reduces adiposity [4,11,12], we hypothesized that increased BAT activity in miR-22 lacking mice fed a HFD may be contributing to higher energy expenditure and reduced overall WAT mass observed in these mice. In addition, we found that BAT of obese miR-22 KO mice displayed increased adrenergic signaling. Collectively, our findings suggest that several mechanisms may be involved in enhanced BAT activity observed in miR-22 KO mice irrespective of diet. However, further studies are needed to examine other potential mechanisms that may be involved in the increased BAT activity under HFD, as well as whether miR-22 can function as a systemic factor to regulate whole body energy homeostasis.

Although miRNAs are highly conserved among species, they can have different targets and play different effects depending on the species. A recent study showed that administration of miR-22-3p antagomir reduced fat mass, liver steatosis and insulin resistance in male mice fed a HFD [59]. Therefore, further investigations are required to determine whether inhibition of miR-22 in human adipose cells and obese patients may play a protective role against obesity-associated metabolic dysfunction, and whether this effect may be mediated by changes in white and brown fat. It is important to note that, in this study, we used whole-body miR-22 KO mice. Whether adipocyte-specific miR-22 deletion in mice will have similar effects compared to miR-22 global KO mice requires future investigation or, alternatively, miR-22 can function through a systemic manner and the beneficial effects that we observed is a combination of miR-22 deletion in both WAT, BAT and any potential effective tissues.

5. Conclusions

In this study, gain- and loss-of-function approaches in vivo and in vitro revealed that deletion of miR-22 is able to exert multiple beneficial effects by targeting different types of adipocytes. Deletion of miR-22 reduces the white adipogenic program, induces WAT browning and enhances BAT function in obese mice. Therefore, our findings support the notion that inhibition of miR-22 might be a novel therapeutic strategy for reducing WAT expansion and enhancing BAT function to combat obesity-related metabolic disorders.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.metabol.2021.154723.

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CRediT authorship contribution statement

V.M.L. and G.P.D. designed the study, the protocols and wrote the manuscript. V.M.L. performed experiments and analyzed data. J.L. designed 3T3L1 CRISPR/Cas9 assay. B.B.B. and M.A.M. performed PIBA and SAT cells extraction and immortalization. C.A.L., C.S.B.S. and C.C. performed animal treatment. M.A.C.R. and T.E.O. performed mitochondrial experiments. C.C.R. and D.P.F. performed PET/CT images. Z.P.H. generated miR-22 null mice. X.H., M.L.B.C., J.C.B.F., W.T.F., M.A.M., C.R.K., and D.Z.W. contributed with protocols and critical revision of the paper. All authors reviewed the manuscript, and approved the final version.

Declaration of competing interest

The authors declare no conflict of interest.

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