Elevated RIF1 Participates in the Epigenetic Abnormalities of Zygotes by Regulating Histone Modifications on MuERV-L in Obese Mice

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Abstract

Background

Maternal obesity impairs embryonic developmental potential and has significantly increases metabolic risks in offspring. However, the epigenetic transmission mechanism of maternal metabolic abnormalities is still poorly understood.

Methods

We induced an obesity model in female mice by high-fat diet (HFD) feeding. The effects of a HFD on the developmental potential of oocytes and embryos, metabolic phenotype, and epigenetic modifications were investigated. The efficacy of metformin administration was assessed. Finally, the regulatory pathway of epigenetic remodeling during zygotic genome activation (ZGA) was explored.

Results

Maternal HFD significantly impaired glucose tolerance in F0 mice, and increased the risk of metabolic disorders in F1 mice. Maternal HFD also decreased embryonic developmental potential, increased ROS and γ H2AX, and reduced the mitochondrial membrane potential (MMP) within oocytes, causing high levels of oxidative stress damage and DNA damage. Starting with this clue, we observed significantly increased RIF1 and shortened telomeres in obese mice. Significant abnormal DNA methylation and histone modification remodeling were observed during ZGA in obese mice, which may be coregulated by RIF1 and the ZGA marker gene MuERV-L. Metformin treatment reduced RIF1, and partially improved ZGA activation status by rescuing epigenetic modification remodeling in oocytes and preimplantation embryos of obese mice. RIF1 knockdown experiments by Trim-Away methods showed that RIF1 degradation altered the H3K4me3 and H3K9me3 enrichment and then triggered the MuERV-L transcriptional activation. Moreover, the ChIP-seq data analysis of RIF1 knockout also showed that RIF1 mediates the transcriptional regulation of MuERV-L by changing the enrichment of H3K4me3 and H3K9me3 rather than DNA methylation.

Conclusion

Elevated RIF1 in oocytes caused by maternal obesity may mediate abnormal embryonic epigenetic remodeling and elevated metabolic risk in offspring by regulating histone modifications on MuERV-L. Metformin treatment can partially rescue the abnormal epigenetic remodeling and prevent epigenetic transmission of maternal metabolic abnormalities.

Introduction

Obesity has become a global epidemic and a worldwide public health concern. All kinds of diseases caused by obesity seriously affect people's health. Women of reproductive age are also challenged by obesity (Ng et al., 2014). Due to the pregnancy and offspring factors, more health problems and potential risks need to be considered in women of reproductive age. Obesity is not only associated with increased risks of almost every common complication of pregnancy, but also plays a direct role in the transgenerational transmission of an obesity or diabetes risk. Increasing evidence suggests that offspring of obese mothers are at increased risk of obesity (Bariani et al., 2020; Keleher et al., 2018), impaired glucose tolerance (Dunn and Bale, 2009; Godfrey et al., 2017), and other facets of the metabolic syndrome (Catalano and Ehrenberg, 2006; Volpato et al., 2012). The increased risks of metabolic abnormalities in offspring are probably associated with epigenetic abnormalities in maternal oocytes (Ou et al., 2019). However, it is not understood how these epigenetic abnormalities in the maternal gametes are passed on to embryos, considering the extensive erasure and reconstruction of epigenetic modifications after zygotic genome activation (ZGA) (Eckersley-Maslin et al., 2018; Xia et al., 2019). Therefore, there is a growing need to understand how maternal diet influences embryo development and offspring health.

Endogenous retroviruses (ERVs) are important components of the mammalian genome (Mager and Stoye, 2015). Transcriptional activation of ERVs occurs during the transition from maternal control to zygotic genome control, signifying the onset of ZGA (Kigami et al., 2003). The murine endogenous retrovirus-like (MuERV-L) gene is an ERV3 family member, which accounts for 80% of recognized long terminal repeat (LTR) element copies predating the human-mouse speciation (Bénit et al., 1999). A significant number of 2-cell genes are initiated by the activation of MuERV-L (Macfarlan et al., 2012), suggesting that MuERV-L is one of the earliest transcribed genes in mouse one-cell embryos (Kigami et al., 2003). MuERV-L participates in rewiring gene expression networks during epigenetic reprogramming (Fu et al., 2019). MuERV-L can be silenced by various epigenetic regulators, including various material factors, histone H3 variants, H3K9 methyltransferases, and histone chaperones (Chen et al., 2020; Maksakova et al., 2013; Rowe et al., 2010). Therefore, we propose that MuERV-L can serve as an important checkpoint to study the epigenetic transgenerational mechanism during the maternal-to-zygotic transition (MZT).

Maternal factors may affect ZGA (Bultman et al., 2006; Wu and Dean, 2020), mediated through MuERV-L. Which maternal factor plays a regulatory role under the influence of obesity is still a mystery. A recent study identified that Rap1-interacting factor 1 (RIF1) shows the strongest effect among a list of novel ERVs regulators (Li et al., 2017). RIF1 was originally discovered as a factor involved in telomere length homeostasis (Hardy et al., 1992). The classical roles of RIF1 are related to DNA damage response (Di Virgilio et al., 2013; Zimmermann et al., 2013), and DNA replication timing (Cornacchia et al., 2012; Yamazaki et al., 2012), indicating a relationship with oxidative stress. Studies have also indicated that it has important roles in embryonic stem cell (ESC) maintenance (Loh et al., 2006) and epigenetic gene regulation (Dan et al., 2014; Li et al., 2017). Emerging studies have highlighted the relationship between metabolic diseases and telomere length (Cheng et al., 2021; Vidacek et al., 2017), indicating that environmental stress affects the regulation of telomeres and RIF1. However, it has not been reported

whether the RIF1 levels of oocytes are changed and further cause epigenetic abnormalities during ZGA in a model of obesity. Accordingly, we propose that RIF1 may be a maternal epigenetic regulator during ZGA in a model of obesity.

Despite the evidence above, the epigenetic transgenerational mechanism of metabolic-related abnormalities is not clear. Here, we attempted to explore the role of ERVs in participating epigenetic changes during the MZT of HFD-fed mice. Additionally, we investigated whether RIF1 is involved in regulating ERVs activity and its epigenetic function.

Materials And Methods

Animals, modeling, and protocols

Three- to four-week-old C57BL/6J mice were purchased from Hunan SJA Laboratory Animal Co., Ltd (Changsha, China) and were housed in the Laboratory Animal Center of Shantou University Medical College (conditions: a 12-h light/dark cycle, 21 ± 2 °C, SPF degree). All animal experiments fit the guidelines of the Medical Animal Care & Welfare Committee and were approved by the Laboratory Animal Ethics Committee of Shantou University Medical College (No. SUMC2020-383).

After adaptive feeding for 1 week, 4- to 5-week-old female mice were randomly divided into the high-fat diet (HFD) and control diet (CD) groups. Mice were fed either a HFD (H10060; HFK Bioscience, Beijing, China; containing 60% fat, and 20% protein by energy ratio) or a control diet (provided by the Laboratory Animal Center of SUMC; containing 10% fat and 20% protein by energy ratio) for 8 weeks. Body weight was measured weekly during the 2 months.

In the research involving F1 generation mice, estrus or pro-estrus HFD and CD female mice were mated to CD male mice. The time of pregnancy was determined by visual inspection of the vaginal plug, which was defined as day 0 of pregnancy. Females were switched to CD at the time of mating. After weaning on day 21, offspring weight was recorded, and female pups were either fed a CD or HFD and weighed weekly (the CD and HFD compositions were the same as those fed to the mothers).

Metformin intervention experiments

To clarify whether the epigenetic abnormalities induced by obesity could be reversed, metformin was used for a further intervention, according to the recommended doses in the previous studies (Abizadeh et al., 2020; Huang et al., 2015; Kamalipour et al., 2020). Briefly, 1000 mg of metformin was dissolved in 20 ml of saline and stored at 4 °C. Modeled mice were divided into the metformin and saline groups. Every mouse received oral gavage administration of 500 mg/kg/day metformin (No. D150959, Sigma, USA) or corresponding normal saline (0.9% NaCl) for 20 consecutive days. Mice were weighed weekly to adjust the administered doses. After the completion of treatment, the mice were sacrificed for various subsequent experiments.

Trim-Away degradation of RIF1 by electroporation

Trim-Away is a new method of degrading endogenous proteins by recruiting endogenous or exogenous TRIM21 to the antibody-bound target proteins (Clift et al., 2018). Protein degradation by Trim-Away is acute and rapid, with half-lives of ~10–20 min (Clift et al., 2017; Clift et al., 2018). To further test whether a decrease in RIF1 would induce subsequent epigenetic changes during ZGA, we employed an electroporation fashion to induce endogenous RIF1 protein reduction. In detail, metaphase II (MII) oocytes were incubated in the acidic Tyrode's solution to digest approximately 30% of the zona pellucida, which typically took 20–60 s. Subsequently, the oocytes were washed in M2 medium three times, and then pipetted into a 1-mm electroporation cuvette (Bio-Rad, catalog no. 1652089) with 20 µl Opti-MEM containing RIF1 antibody. The BioRad Gene Pulser XCell electroporator was used. Two square wave pulses were applied (voltage of 30 V, 5-ms pulse length, and 100-ms pulse interval) according to the published protocol (Maas et al., 2018). Immediately after electroporation, the zygotes were retrieved washed in M2 medium three times, and then cultured in KSOM (M1450; Aibei Biotechnology, China) for 1 h. Finally, the conventional in vitro fertilization procedures were applied.

Glucose tolerance tests and insulin tolerance tests

For glucose tolerance tests (GTTs), 20% glucose (2 g/kg body weight) was fed by intragastrically administered after an overnight fasting. Blood glucose levels were measured using an Yuwell fasting glucometer (No.580, Yuyue Medical Equipment Co., Ltd, China). Blood was obtained from the tail vein, and glucose levels were monitored at 0, 15, 30, 60, 90, and 120 min after 2 months of feeding with a CD or HFD.

After one week of recovery from the GTTs, insulin tolerance tests (ITTs) were then applied in which mice were injected intraperitoneally with insulin (1 IU/kg body weight, Fosun Pharma Co., Ltd, China). Blood samples were collected and measured by the Yuwell fasting glucometer at 0, 15, 30, 60, 90 and 120 min after injection.

Oocyte and embryo collection

To collect MII oocytes, female mice were superovulated by injecting 10 IU pregnant mare serum gonadotropin (PMSG) followed by 10 IU human chorionic gonadotropin (hCG) 48 h later and were sacrificed by cervical dislocation 14 h post-hCG injection (p-hCG). Cumulus-oocyte complexes (COCs) were released from the oviduct ampulla region. When fertilization was not required, COCs were transferred into medium containing 0.5 mg/ml hyaluronidase at 37 °C to digest cumulus granulosa cells for harvesting denuded MII oocytes.

For collecting embryos at each stage, in vitro fertilization and embryo culture were applied according to our previously published protocols (Huang et al., 2019). Briefly, spermatozoa, obtained from the cauda epididymis of CD male mice, were incubated in capacitation medium (HTF medium containing 1.5% BSA) at 37 °C in a 5% CO₂ incubator for 1 hour. Then, capacitated spermatozoa were added to a pre-prepared fertilization droplet (HTF medium containing 0.4% BSA) with COCs. After 6 hours of fertilization, zygotes

were either collected for subsequent experiments, or transferred into KSOM medium overlaid with mineral oil for collecting embryos at each stage.

Reactive oxygen species and mitochondrial membrane potential

To detect the level of reactive oxygen species (ROS) and the mitochondrial membrane potential (MMP) in oocytes and zygotes, dihydroethidium (S0063, Beyotime Biotechnology, Shanghai, China) and JC-1 kits (C2006, Beyotime Biotechnology, Shanghai, China) were applied respectively. According to our previous protocols (Li et al., 2019), a stock solution of dihydroethidium (1×10^{-3} mol/L in DMSO) was added to the M2 medium to a final concentration of 10 μ mol/L, and JC-1 was diluted in PBS to a final concentration of 1.25 μ mol/L. Fluorescence staining was detected immediately under a fluorescence microscope (Nikon Eclipse 90 Ni-E). ImageJ (NIH Image, Bethesda, MD) was used to quantify the fluorescence intensity.

Western blot

Western blotting was performed as described previously (Bariani et al., 2020; Han et al., 2018). A total of 100 MII oocytes or embryos were added to 2 $\times$ SDS sample buffer, incubated at 95 °C for 5 min and then frozen at -30 °C until further use. Protein samples were electrically separated by SDS-PAGE. Membranes were incubated at 4 °C overnight with the following primary antibodies: rabbit anti-PPAR γ antibody (1:1000; CST, #2443), mouse anti-SIRT3 antibody (1:1000; Santa Cruz, sc-365175) or rabbit anti-GAPDH antibody (1:5000; Sigma, A5441). The appropriate species-specific horseradish peroxidase (HRP)-conjugated secondary antibodies (1:5000; Thermo Fisher Scientific, 31460 and 31470) were applied for a 1-h incubation at room temperature. Signals were measured using ECL western blotting substrate (4A Biotech, China) according to the manufacturer's instructions.

Quantitative real-time PCR.

One hundred oocytes or embryos were collected from each group. RNA was extracted according to the manual of the PicopureTM RNA Isolation Kit (KIT0204, Thermo, USA). The concentration and quality of RNA were measured by a NanoDrop ND-2000 (Thermo, USA). cDNA synthesis was conducted using the PrimeScript First-Strand cDNA Synthesis Kit (6110A, Takara, Japan). Real-time PCR was performed with Takara RR420Q TB Green[®] Premix Ex Taq[™] (RR420Q, Takara, Japan) in a Real-Time PCR Detection System (CFX96, Bio-Rad, USA). GAPDH was used as an internal control for each sample. The relative mRNA expression levels of target genes were calculated using the $2^{-\Delta\Delta C_t}$ method. Primers for qPCR were synthesized by GenePharma (GenePharma Co., Ltd, China) and are listed in Supplementary Table 1.

Immunofluorescence (IF)

To detect a single target, denuded oocytes or embryos were digested with Tyrode's solution to remove the zona pellucida, fixed with 4% paraformaldehyde for 30 min, and then permeabilized with PBS with 0.5% Triton X-100 for 20 min. After blocking in PBS containing 3% BSA and 10% fetal bovine serum for 1 h,

samples were incubated with primary antibody at 4 °C overnight, followed by Alexa Fluor 488–conjugated goat anti-rabbit or Alexa Fluor 555–conjugated goat anti-mouse secondary antibodies at room temperature for 1 hr. The primary antibody used included RIF1 antibody (1:200; RayBiotech), MuERV-L Gag antibody (1:200; AF0240, Beyotime), H3K4me3(1:200; ab1012, Abcam), H3K9me3(1:200; Abcam), anti-γ-H2AX antibody (1:300; Abcam, ab22551), anti-PPARγ antibody (1:1000; CST, #2443), and anti-SIRT3 antibody (1:1000; Santa Cruz, sc-365175).

For detecting 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC), immunofluorescence double staining was applied as described previously (Han et al., 2018). Briefly, the procedures before primary antibody immunostaining were the same as those for other targets. After blocking, samples were stained overnight at 4 °C with rabbit polyclonal anti-5hmC antibody (1:200; Active Motif, 39769) and mouse monoclonal anti-5mC antibody (1:200; Epigentek). The cells were washed and incubated with Alexa Fluor 488–conjugated goat anti-rabbit and Alexa Fluor 555–conjugated goat anti-mouse secondary antibodies (1:150; Molecular Probes) for 4 h at room temperature.

After incubation with secondary antibodies, Antifade Mounting Medium containing DAPI (AAPR11-A5, China) was applied to cover the slides. Fluorescence staining was detected immediately under a fluorescence microscope (Nikon Eclipse 90 Ni-E). ImageJ (NIH Image, Bethesda, MD) was used to quantify the fluorescence intensity. To ensure comparability, identical conditions, including the same confocal microscope settings, were used for CD or HFD samples.

Telomere measurement by quantitative real-time PCR

The relative telomere length (RTL) of oocytes was measured from the total genomic DNA using qPCR as previously described (Cheng et al., 2013). Briefly, 60 oocytes of each group were collected. DNA was extracted using the QIAmp DNA micro Kit (Qiagen 56304, Valencia, CA, USA). The telomere signal was normalized to the signal from the single-copy gene to generate a relative telomere to single copy gene (T/S) ratio indicative of the RTL. A mouse 36B4 single-copy gene was used as the reference control gene. Equal amounts of DNA (300 pg) were used for each reaction. Primers for qPCR are listed in Supplementary Table 1.

Statistical Analysis

Data are presented as the mean values $\pm$ SD. $P < 0.05$ was considered statistically significant. For statistical comparisons, ANOVA or Student's t test was used in SPSS17.0 software. GraphPad Prism 9.0 was used to draw cartograms. All experiments were repeated three times with a similar result, and representative results are shown in this article. ChIP-seq data were obtained from GEO database. The Integrative Genomics Viewer (IGV) browser was used to display ChIP-seq data.

Results

Maternal HFD increased the risk of offspring metabolic disorder

As shown in Figure 1, an 8-week of HFD induced significant obesity, and increased the abdominal fat accumulation (Fig. 1A and 1B). We found a significant difference in body weight emerged after one month of high-fat feeding between the groups (Fig. 1C). To understand whether a HFD leads to obesity in F1 offspring, we further compared the F1 body weight. The results showed that there was no significant difference in F1 body weight under a CD (Fig. 1D). Furthermore, when a HFD was administered, and we found that the F1 offspring fed a parental HFD had a significantly higher weight than the F1 offspring fed a parental CD (Fig. 1E).

To determine the effect of a HFD on glucose metabolism, we conducted GTTs and ITTs. The results of the GTTs showed that higher blood glucose and delayed peak values appeared in the HFD group (Fig. 1F and 1G). Although there was no significant difference in F1 weight under a normal diet (Figure 1D), the GTTs and ITTs profiles showed significant differences in F1 mice compared to those fed a CD (Fig. 1H and 1I).

These results suggested that the HFD induced significant obesity and impaired glucose tolerance in F0 mice. Although F1 mice showed no significant difference in body weight, impaired glucose tolerance has been existed and further HFD feeding could induce a significant difference in body weight.

HFD leads to poor oocyte and preimplantation embryo results

We first evaluated the morphology and developmental potential of the oocytes and preimplantation embryos of each group. Our results showed that the average number of MII oocytes in each mouse showed no significant difference between the HFD and CD groups (16.86 ± 3.23 vs. 18.18 ± 4.57 , $P = 0.06$) (Fig. 2A). Moreover, the percentages of 2 cell and blastocyst formation were significantly lower in the HFD group than in the control group (0.653 ± 0.042 vs. 0.875 ± 0.049 and 0.448 ± 0.025 vs. 0.598 ± 0.072 respectively) (Fig. 2A), with more deaths or cytoplasmic fragmentation (red asterisk).

We next examined the level of oxidative stress and mitochondrial function in oocytes of the HFD and CD groups. We used a DHE probe to detect intracellular ROS levels in oocytes in the HFD and CD groups. As shown in Fig. 2B, the fluorescence intensity ratios of DHE in MII oocytes from the HFD group (2.79 ± 0.61) were significantly higher than those of the CD group (0.37 ± 0.10 , $P < 0.001$). Similarly, a JC-1 probe was used to detect MMP in oocytes, partly reflecting the functional status of the mitochondria. The results showed that the red/green fluorescence intensity ratios of JC-1 were significantly stronger in the CD group (7.298 ± 2.00) than those in the HFD group (0.62 ± 0.11 , $P < 0.001$, Fig. 2C), indicating a higher MMP.

Finally, we evaluated the energy metabolic function of the oocytes in the HFD and CD groups. PPAR γ and SIRT3 were the most important candidates in the energy metabolic pathway (Gross et al., 2017; Wang et al., 2019). Therefore, the expression levels of PPAR γ and SIRT3 in oocytes and preimplantation embryos were illustrated by IF, WB and qPCR. The IF results revealed that HFD caused the deficiencies in PPAR γ and SIRT3 levels in maternal oocytes (Fig. 2D), and these abnormalities in energy metabolism also occurred in the blastocysts (Fig. 2D), implying that they represent a precursor to metabolic

abnormalities in offspring genes. As a validation of these findings, the results from WB and PCR also showed similar trends (Fig. 2E and 2F).

Accordingly, these results suggested that a HFD induced high levels of oxidative stress and impaired mitochondrial function and energy metabolism pathways in oocytes, which consistently persisted into the preimplantation embryos and ultimately led to abnormal developmental potential of embryos and impaired energy metabolic function.

Elevated RIF1 and epigenetic modification changes in oocytes and preimplantation embryos of obese mice

ZGA is controlled by stored maternal proteins (Wu and Dean, 2020), which are influenced by the prepregnancy environment and lifestyle factors. RIF1, as a negative regulator of telomere elongation, is involved in the DNA oxidative damage response. We explored whether the oxidative damage induced by a HFD altered the levels of RIF1 and the RTL within the oocytes. We found that the expression of RIF1 was localized to the nucleus and that fluorescent staining was low in CD oocytes (Fig. 3A). The average fluorescence intensity of RIF1 was significantly stronger in HFD oocytes than in CD oocytes (marked with green arrows, Fig. 3A). The qPCR results also confirmed a significant higher expression of RIF1 in HFD oocytes (the middle bar chart in the bottom of Fig. 3A). We inferred that the elevated RIF1 might be associated with the shortened telomeres and elevated γ H2AX caused by the HFD. As shown in the right bar chart of Fig. 3A, the qPCR results indicated that the RTL in HFD oocytes was substantially shortened by 50% ~ 60% compared with that in CD oocytes. Additionally, γ H2AX, a biomarker of DNA double-strand breaks, were significantly higher in the HFD group than in the CD group ($P < 0.01$, Fig. 3B). MuERV-L, an embryonic totipotency marker, was evaluated by IF and qPCR. IF staining showed that MuERV-L gag was detectable from the stage of 2-cell embryos stage (Fig. 3C). Surprisingly, the qPCR results revealed that the expression of MuERV-L was most apparent in zygotes and 2-cell embryos (the right line chart at the bottom of Fig. 3C), which was quite different from the IF results. Whether HFD induced differences in epigenetic modifications during ZGA is unknown. Both DNA methylation and histone modifications were investigated in this study. We found that the average fluorescence intensities of 5mC in HFD oocytes were significantly lower than those in the CD group (Fig. 3D). Moreover, a significant reduction in the 5mC fluorescence intensity and a significant increase in the 5hmC fluorescence intensity were found in the maternal pronuclear (PN) of HFD zygotes at 7 h postinsemination (hpi) compared with the levels of CD zygotes (Fig. 3D). However, no significant differences in 5mC and 5hmC were found in the paternal PN region of zygotes and 2-cell embryos (Fig. 3D). Similar to the DNA methylation results, asymmetric epigenetic establishments in zygotes also existed with respect to histone modifications. In detail, we also found that a significant reduction in the H3K4me3 fluorescence intensity and a significant increase in the H3K9me3 fluorescence intensity were found in the maternal PN region of HFD zygotes at 7 hpi compared with CD zygotes (Fig. 3E). The fluorescence intensity of H3K4me3 from oocytes to blastocysts in the HFD group was always significantly lower than that in the control group ($P < 0.05$, Fig. 3E), whereas the results were reversed from H3K9me3 ($P < 0.05$, Fig. 3E). Taken together, these results showed that obesity induced an elevated RIF1, the abnormal totipotency of zygotes, and the asymmetric epigenetic

remodeling of both DNA methylation and histone modifications. These epigenetic abnormalities may be responsible for the increased risks of metabolic abnormalities in the offspring.

Metformin treatment reduced RIF1 and modified the epigenetic modification changes in oocytes and preimplantation embryos of obese mice

Given the above abnormalities of metabolism, DNA stress, and epigenetic modifications caused by obesity, we further explored whether these abnormalities could be reversed by metformin, an oral insulin-sensitizing agent that is widely used in the treatment of patients with type II diabetes, metabolic syndrome and polycystic ovary syndrome. After 20 days of metformin intervention, the disruptions of both ROS and MMP in oocytes were partially rescued (Fig. 4B and 4C). Additionally, metformin treatment significantly reduced RIF1 (top images and middle bar chart of Fig. 4E). However, no significant difference was found in the TFL ($P = 0.14$, bottom bar chart of Fig. 4E). As RIF1 was related to the DNA damage response, we evaluated the intensity of γ H2AX again and found that metformin partially counteracted the elevated γ H2AX caused by the HFD (Fig. 4D). The expression of MuERV-L detected by IF (top images and middle bar chart of Fig. 4F) and qPCR (bottom bar chart of Fig. 4E) all showed a significant amelioration, indicating an improvement in the embryo development potential by the administration of metformin. Unexpectedly, metformin administration did not improve the MII rate of oocyte, 2-cell rate, and the 8-cell rate ($P = 0.73$, $P = 0.85$ and $P = 0.15$, respectively; Fig. 4A). As shown in Fig. 4G, when metformin was administered, the loss of DNA methylation in the oocytes and in the maternal PN region of HFD zygotes were both significantly improved ($P = 0.02$ and $P < 0.001$, respectively). Correspondingly, the 5hmC in the oocytes and the maternal PN region of HFD zygotes were significantly reduced ($P = 0.04$ and $P < 0.001$, respectively). However, metformin administration did not alter the abnormalities in 5mC and 5hmC in the 2-cell embryos ($P > 0.05$, Fig. 4G). In contrast to the variation tendency of DNA methylation, abnormalities in histone modifications, including H3K4me3 and H3K9me3, were significantly modified from the oocytes to the blastocyst embryos (all $P < 0.05$, Fig. 4H). In other words, the impact of metformin intervention had a stronger effect on histone modifications than on DNA methylation.

Altogether, the findings above indicated that metformin administration was associated with some improvements in oocyte antioxidant activity, DNA damage, ZGA and epigenetic remodeling, although no significant improvements in the blastocyst formation rate were observed.

Knockdown of RIF1 changes the totipotent ability and epigenetic modification of zygotes during ZGA.

To clarify whether the elevated RIF1 in HFD oocytes mediated the developmental abnormalities and epigenetic remodeling in the embryos, we performed knockdown experiments by electroporating an anti-RIF1 antibody into zygotes, which induced a transient decrease in RIF1 protein levels based on the Trim-Away principle by binding Trim21. First, the effectiveness of electroporation was validated by mCherry-Trim21 mRNA, which allows visualization of Trim21 in the living cells via red fluorescence. Visible fluorescent mCherry-Trim21 protein could be captured when mCherry-Trim21 mRNA was added into the electroporation reagents (Fig. 5A). Next, various concentrations of Trim21 and RIF1 antibody were tested

(Fig. 5A). However, we found that when cotransferred exogenous Trim21 and RIF1 antibody into the oocytes, it would lead to a persistent deficiency of endogenous RIF1, which ultimately led to embryonic arrest. Even if we used mCherry-Trim21 mRNA at a concentration of 100 ng/μl, which was already lower than the reported concentration (Gerri et al., 2020). Therefore, only RIF1 antibody was electroporated into the cell, and endogenous RIF1 was slightly knocked down by endogenous Trim21. Finally, 10 ng/μl of RIF1 antibody was selected as an effective concentration, which not only ensured continued embryo survival but also significantly reduced endogenous RIF1 (line chart at the bottom of Fig. 5A).

Using the concentrations outlined above, the functional consequences were assessed. We confirmed that the protein level of endogenous RIF1 was reduced when 10 ng/μl of RIF1 antibody was electroporated into oocytes (Fig. 5B). Moreover, the effects of RIF1 knockdown on ZGA were evaluated. As shown in Fig. 5C, knockdown of RIF1 elevated the expression level of MuERV-L, as validated by both IF and qPCR. Unexpectedly, we found that the knockdown of RIF1 did not alter DNA methylation levels from the oocytes to 2-cell embryos in HFD mice (Fig. 5D). However, global increased enrichment of H3K4me3 and decreased enrichment of H3K9me3 were also observed in the maternal PN region of RIF1 knockdown zygotes by IF (Fig. 5E). To further clarify the enrichment changes in the histone modifications of the promoter region of MuERV-L, we analyzed the RIF1 ChIP-seq data in recent public datasets (GSE73952, GSE98149, and GSE98256). We first examined the common histone modifications at all stages from oocytes to blastocysts and found that the enrichment of H3K27me3 on the MuERV-L was not strong from during ZGA (Fig. 5F). That is, the H3K4me3 and H3K9me3 histone modifications play a better role in the regulating the transcription of MuERV-L than H3K27me3 during ZGA. This was also consistent with the results of our main investigations of H3K4me3 and H3K9me3 in this study. Furthermore, the knockdown of RIF1 did not cause changes in H3K27me3 enrichment on MuERV-L, but rather caused significant changes in H3K4me3 and H3K9me3 enrichment (Fig. 4G), which was highly consistent with the results obtained by Trim-Away (Fig. 4E). ATAC-seq showed that the knockdown of RIF1 significantly increased the mRNA transcription and genome accessibility around RIF1-bound ERV loci (Fig. 4G), indicating an enhanced activation of ZGA.

Altogether, these data suggested that RIF1 mediated the assembly of histone modifications on the MuERV-L and that elevated RIF1 induced by the maternal obesity could facilitate ZGA transcriptional activity and epigenetic remodeling by regulating the histone modifications of MuERV-L.

Discussion

In this study, we comprehensively conducted a detailed phenotypic assessment of oocytes and preimplantation embryos induced by the maternal obesity, and identified that RIF1 could act as a maternal epigenetic factor related to DNA stress damage and could mediate epigenetic remodeling in obese mice. Furthermore, metformin administration reduced abnormally elevated RIF1 and improved epigenetic remodeling. Finally, we demonstrated that overexpression of RIF1 can mediate metabolic phenotypic differences by regulating epigenetic modifications on MuERV-L (Figure 6).

We confirmed that RIF1 was significantly elevated in oocytes of HFD mice when compared with those of CD mice. RIF1 was first identified as a telomere-binding protein in yeast that negatively regulated telomere length (Hardy et al., 1992). Later studies identified its important roles in the DNA damage response (Di Virgilio et al., 2013; Zimmermann et al., 2013) and DNA replication timing (Cornacchia et al., 2012; Yamazaki et al., 2012). A recent study showed that RIF1 interacts with H3K9 methylation to mediate heterochromatic silencing, which indicates its roles in epigenetic gene regulation (Dan et al., 2014). These studies have fully demonstrated that RIF1 is a multifunctional protein.

Obesity is a worldwide health problem, involving multiple abnormal phenotypes such as inflammation, oxidative stress, DNA damage, and mitochondrial dysfunction in oocytes (Andreas et al., 2019; Snider and Wood, 2019). An inactive lifestyle, such as obesity, type 2 diabetes mellitus and cardiovascular disease, is associated with short telomeres (Cheng et al., 2021). In our study, we found that HFD induced elevated ROS and γ H2AX, and impaired mitochondrial function in oocytes. Furthermore, the fluorescence intensity of telomeres indicated that telomeres were shorter in HFD mice than in CD mice. These abnormal phenotypes ultimately caused the elevated RIF1 within the oocytes. These results suggest that RIF1 is an obesity-associated maternal factor.

Before ZGA, zygotes are regulated by maternal factors, including protein and RNA stored in oocytes (Lee et al., 2014). Maternally provided pioneer transcription factors may initiate ZGA, by inducing the opening of chromatin and recruiting other transcriptional machinery (Lee et al., 2014). Protein profiles stored within the oocytes were influenced by multiple factors including the maternal metabolic environment, aging factors, and oxidative stress. Different levels of maternal proteins, acting as epigenetic factors, regulated the activation of ZGA genes and the accessibility of chromatin. In this study, our results showed that elevated RIF1 was accompanied by decreased expression of MuERV-L, and impaired establishment of epigenetic asymmetry including DNA methylation and histone modifications. Moreover, treatment with metformin administration significantly reduced RIF1, and improved ZGA status and epigenetic remodeling. Therefore, RIF1, as a maternal factor, may be involved in epigenetic remodeling.

To further support the main role of RIF1 in regulating ZGA, the Trim-Away experiments and Chip-seq data in this study illustrated that RIF1 engaged in modulating the activation state of MuERV-L by interacting with and facilitating H3K4me3 and H3K9me3, but not H3K27me3. Knockdown of RIF1 further caused the transcriptional activation of MuERV-L and the remodeling of histone modifications. It is worth noting that knockout of RIF1 leads to failure in embryonic developmental failure. Therefore, ensuring further developmental potential is critical when endogenous RIF1 is degraded. Trim-Away, as a recent technique, degrades endogenous proteins guided by antibodies binding with TRIM21 (Clift et al., 2017). Unlike DNA- or RNA-targeting methods, which take hours or days to deplete proteins of interest, the Trim-Away system removes endogenous proteins within minutes (Di Virgilio et al., 2013). Since the initial activation of ZGA occurs within a few hours after fertilization, we proposed that Trim-Away was the best method to study transient protein depletion during ZGA. Consistent with our study, some researchers have also reduced endogenous transcription factors in mouse zygotes and embryos by the Trim-Away assay (Gerri et al., 2020; Israel et al., 2019; Mehlmann et al., 2019). Although a study demonstrated that RIF1 promotes a

repressive chromatin state to safeguard against ERVs activation, and its depletion significantly enhances reprogramming efficiency. In addition, RIF1 not only facilitates H3K9me3 and H3K27me3 occupancy at ERVs but also interacts with the histone marks (Vermeulen et al., 2010), either directly or indirectly via Chaf1a. A recent study also illustrated that RIF1 knockout causes global alterations of compartments and epigenetic states (Klein et al., 2021). However, these results were investigations at the embryonic stem cell level, which did not fully represent the changes in zygotes. Our study precisely illustrated the role of RIF1 in the mouse zygote. Hence, it is conceivable that RIF1 is an essential factor during ZGA but that abnormally elevated RIF1 caused by DNA damage will affect the transcriptional activation of MuERV-L and the deposition of histone modifications on MuERV-L.

In this study, we identified that MuERV-L expression began at the at 6 hpi at the 1-cell stage and reached its peak at the 2-cell stage (Fig. 3B). Moreover, we found that MuERV-L, as an acting target element of RIF1, is a core element that initiates ZGA. The activation state of MuERV-L also significantly affected the genome-wide enrichment of epigenetic remodeling (Fig. 3C, 3D and 3E). These results sufficiently suggested that MuERV-L appeared to be an important regulatory element of epigenetic modifications. Concordant with our results, MuERV-L has been reported to be highly expressed in 2-cell embryos and declines sharply at the later stages (Kigami et al., 2003), showing a close relationship with open chromatin (Wu et al., 2016). Chromatin accessibility is important for the control of selective gene expression. MuERV-L is one of the earliest transcribed genes in mouse 1-cell embryos (Kigami et al., 2003), and promotes the expression of hundreds of neighboring genes (Schoorlemmer et al., 2014). Other studies underlined that the expression of MuERV-L was regulated by some maternal factors such as REX1 (Schoorlemmer et al., 2014), TRPS1 (Liu et al., 2019), and DPPA3 (Huang et al., 2017), contributing to the epigenetic plasticity of mouse embryos. However, our study found that RIF1 was also involved in regulating the transmission of the embryonic metabolic phenotype by regulating MuERV-L. Accordingly, we propose that MuERV-L is an important regulatory element that mediates abnormal epigenetic reconstruction in obese mice.

Limitations

Some limitations should be addressed in this study. First, a single dose of metformin was used in our study. Although a dose of 500 mg/kg was used in some studies and proved to be useful for improving embryonic development, whether there is dose-dependent improvement in epigenetic remodeling by metformin, the relationship between the dose of metformin and RIF1 level and the level at which RIF1 should be controlled still need to be determined, considering that RIF1 depletion leads to early embryonic lethality. Second, due to the difficulty in collecting amounts of preimplantation embryos for histone sequencing experiments (CUT&Tag or ChIP-seq), the histone sequencing data were obtained from the GEO database, which was another major limitation. Finally, it was confirmed in this study that RIF1, as a maternal epigenetic factor, was involved in regulating the epigenetic remodeling of ERVs during ZGA, but whether there are other factors that are jointly involved in this process requires further investigation.

Conclusion

In summary, we concluded, for the first time, that maternal obesity caused an increase in RIF1 in oocytes, resulting in the reduced embryonic developmental potential and epigenetic asymmetry remodeling. which in turn led to an impaired metabolic phenotype of offspring. Finally, we proposed that the elevated RIF1 and those epigenetic abnormalities could be partially rescued by metformin treatment. Indeed, metformin treatment can counteract oxidative stress damage in oocytes and reduce elevated RIF1 caused by DNA damage, which is beneficial for preventing epigenetic transmission of maternal metabolic abnormalities. RIF1 may be a promising candidate for therapeutic intervention.

Abbreviations

HFD: high-fat diet; ZGA: zygotic genome activation; MMP: mitochondrial membrane potential; ERVs: Endogenous retroviruses; MuERV-L: Murine endogenous retrovirus-like; MZT: maternal-to-zygotic transition; RIF1: Rap1-interacting factor 1; ESC: embryonic stem cell; CD: control diet; MII: metaphase II; GTTs: glucose tolerance tests; ITTs: insulin tolerance tests; PMSG: pregnant mare serum gonadotropin; hCG: human chorionic gonadotropin; COCs: Cumulus–oocyte complexes; ROS: reactive oxygen species; IF: immunofluorescence; 5mC: 5-methylcytosine; 5hmC: 5-hydroxymethylcytosine; RTL: relative telomere length; IGV: Integrative Genomics Viewer.

Declarations

Availability of data and materials

The main original data were provided in this manuscript and supplementary files. Any other original data were available from the corresponding author when needed. The ChIP-seq datasets analyzed in this study were previously published and available at the GEO website (GSE73952, GSE98149, GSE98256).

Authors' contributions

JH conceived the initial idea, performed the most experiments, and wrote the manuscript. GR provided considerable technical assistance. JS and LS performed the acquisition, analysis and interpretation of the data. ZL supervised the program, reviewed the manuscript and acquired the funding. All authors have read and approved the final manuscript.

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Consent for publication

Not applicable.

Competing interests

The authors declare they have no conflicts of interest.

References

1. Abizadeh, M., M.G. Novin, F. Amidi, S.A. Ziaei, M.A. Abdollahifar, and H. Nazarian. Potential of Auraptene in Improvement of Oocyte Maturation, Fertilization Rate, and Inflammation in Polycystic Ovary Syndrome Mouse Model. *Reprod. Sci.* 2020, 27:1742-1751.
2. Andreas, E., M. Reid, W. Zhang, and K.H. Moley. The effect of maternal high-fat/high-sugar diet on offspring oocytes and early embryo development. *Molecular human reproduction* 2019, 25:717-728.
3. Bariani, M.V., F. Correa, A.P. Domínguez Rubio, C. Marvaldi, J.A. Schander, J.S. Beltrame, et al. Maternal obesogenic diet combined with postnatal exposure to high-fat diet induces metabolic alterations in offspring. *Journal of cellular physiology* 2020, 235:8260-8269.
4. Bénit, L., J.B. Lallemand, J.F. Casella, H. Philippe, and T. Heidmann. ERV-L elements: a family of endogenous retrovirus-like elements active throughout the evolution of mammals. *J. Virol.* 1999, 73:3301-3308.
5. Bultman, S.J., T.C. Gebuhr, H. Pan, P. Svoboda, R.M. Schultz, and T. Magnuson. Maternal BRG1 regulates zygotic genome activation in the mouse. *Genes & development* 2006, 20:1744-1754.
6. Catalano, P.M., and H.M. Ehrenberg. The short- and long-term implications of maternal obesity on the mother and her offspring. *BJOG : an international journal of obstetrics and gynaecology* 2006, 113:1126-1133.
7. Chen, F., W. Zhang, D. Xie, T. Gao, Z. Dong, and X. Lu. Histone chaperone FACT represses retrotransposon MERV1 and MERV1-derived cryptic promoters. *Nucleic acids research* 2020, 48:10211-10225.
8. Cheng, E.H., S.U. Chen, T.H. Lee, Y.P. Pai, L.S. Huang, C.C. Huang, et al. Evaluation of telomere length in cumulus cells as a potential biomarker of oocyte and embryo quality. *Human reproduction (Oxford, England)* 2013, 28:929-936.
9. Cheng, F., L. Carroll, M.V. Joglekar, A.S. Januszewski, K.K. Wong, A.A. Hardikar, et al. Diabetes, metabolic disease, and telomere length. *The lancet. Diabetes & endocrinology* 2021, 9:117-126.
10. Clift, D., W.A. McEwan, L.I. Labzin, V. Konieczny, B. Mogessie, L.C. James, et al. A Method for the Acute and Rapid Degradation of Endogenous Proteins. *Cell* 2017, 171:1692-1706 e1618.

11. Clift, D., C. So, W.A. McEwan, L.C. James, and M. Schuh. Acute and rapid degradation of endogenous proteins by Trim-Away. *Nat. Protoc.* 2018, 13:2149-2175.
12. Cornacchia, D., V. Dileep, J.P. Quivy, R. Foti, F. Tili, R. Santarella-Mellwig, et al. Mouse Rif1 is a key regulator of the replication-timing programme in mammalian cells. *The EMBO journal* 2012, 31:3678-3690.
13. Dan, J., Y. Liu, N. Liu, M. Chiourea, M. Okuka, T. Wu, et al. Rif1 maintains telomere length homeostasis of ESCs by mediating heterochromatin silencing. *Developmental cell* 2014, 29:7-19.
14. Di Virgilio, M., E. Callen, A. Yamane, W. Zhang, M. Jankovic, A.D. Gitlin, et al. Rif1 prevents resection of DNA breaks and promotes immunoglobulin class switching. *Science* 2013, 339:711-715.
15. Dunn, G.A., and T.L. Bale. Maternal high-fat diet promotes body length increases and insulin insensitivity in second-generation mice. *Endocrinology* 2009, 150:4999-5009.
16. Eckersley-Maslin, M.A., C. Alda-Catalinas, and W. Reik. Dynamics of the epigenetic landscape during the maternal-to-zygotic transition. *Nat. Rev. Mol. Cell Biol.* 2018, 19:436-450.
17. Fu, B., H. Ma, and D. Liu. Endogenous Retroviruses Function as Gene Expression Regulatory Elements During Mammalian Pre-implantation Embryo Development. *International journal of molecular sciences* 2019, 20:
18. Gerri, C., A. McCarthy, G. Alanis-Lobato, A. Demtschenko, A. Bruneau, S. Loubersac, et al. Initiation of a conserved trophectoderm program in human, cow and mouse embryos. *Nature* 2020, 587:443-447.
19. Godfrey, K.M., R.M. Reynolds, S.L. Prescott, M. Nyirenda, V.W. Jaddoe, J.G. Eriksson, et al. Influence of maternal obesity on the long-term health of offspring. *The lancet. Diabetes & endocrinology* 2017, 5:53-64.
20. Gross, B., M. Pawlak, P. Lefebvre, and B. Staels. PPARs in obesity-induced T2DM, dyslipidaemia and NAFLD. *Nat. Rev. Endocrinol.* 2017, 13:36-49.
21. Han, L., C. Ren, L. Li, X. Li, J. Ge, H. Wang, et al. Embryonic defects induced by maternal obesity in mice derive from Stella insufficiency in oocytes. *Nature Genetics* 2018, 50:432-442.
22. Hardy, C.F., L. Sussel, and D. Shore. A RAP1-interacting protein involved in transcriptional silencing and telomere length regulation. *Genes & development* 1992, 6:801-814.
23. Huang, Y., S. Ha, Z. Li, J. Li, and W. Xiao. CHK1-CENP B/MAD2 is associated with mild oxidative damage-induced sex chromosome aneuploidy of male mouse embryos during in vitro fertilization. *Free Radical Biology and Medicine* 2019, 137:181-193.
24. Huang, Y., J.K. Kim, D.V. Do, C. Lee, C.A. Penfold, J.J. Zylicz, et al. Stella modulates transcriptional and endogenous retrovirus programs during maternal-to-zygotic transition. *eLife* 2017, 6:
25. Huang, Y., Y. Yu, J. Gao, R. Li, C. Zhang, H. Zhao, et al. Impaired oocyte quality induced by dehydroepiandrosterone is partially rescued by metformin treatment. *PLoS One* 2015, 10:e0122370.
26. Israel, S., E. Casser, H.C.A. Drexler, G. Fuellen, and M. Boiani. A framework for TRIM21-mediated protein depletion in early mouse embryos: recapitulation of Tead4 null phenotype over three days. *BMC genomics* 2019, 20:755.

27. Kamalipour, F., H. Jalali, and M. Azarnia. Comparison the Effect of Metformin and Clomiphene Citrate on Sirtuin3 gene Expression in the Oocytes of Mice with Polycystic Ovary Syndrome. *Iran J Pharm Res* 2020, 19:160-168.
28. Keleher, M.R., R. Zaidi, S. Shah, M.E. Oakley, C. Pavlatos, S. El Idrissi, et al. Maternal high-fat diet associated with altered gene expression, DNA methylation, and obesity risk in mouse offspring. *PLoS One* 2018, 13:e0192606.
29. Kigami, D., N. Minami, H. Takayama, and H. Imai. MuERV-L is one of the earliest transcribed genes in mouse one-cell embryos. *Biol. Reprod.* 2003, 68:651-654.
30. Klein, K.N., P.A. Zhao, X. Lyu, T. Sasaki, D.A. Bartlett, A.M. Singh, et al. Replication timing maintains the global epigenetic state in human cells. *Science* 2021, 372:371-378.
31. Lee, M.T., A.R. Bonneau, and A.J. Giraldez. Zygotic genome activation during the maternal-to-zygotic transition. *Annual review of cell and developmental biology* 2014, 30:581-613.
32. Li, J., S. Ha, Z. Li, Y. Huang, E. Lin, and W. Xiao. Aurora B prevents aneuploidy via MAD2 during the first mitotic cleavage in oxidatively damaged embryos. *Cell Prolif* 2019, 52:e12657.
33. Li, P., L. Wang, B.D. Bennett, J. Wang, J. Li, Y. Qin, et al. Rif1 promotes a repressive chromatin state to safeguard against endogenous retrovirus activation. *Nucleic acids research* 2017, 45:12723-12738.
34. Liu, Y., S. Xu, X. Lian, Y. Su, Y. Zhong, R. Lv, et al. Atypical GATA protein TRPS1 plays indispensable roles in mouse two-cell embryo. *Cell cycle (Georgetown, Tex.)* 2019, 18:437-451.
35. Loh, Y.H., Q. Wu, J.L. Chew, V.B. Vega, W. Zhang, X. Chen, et al. The Oct4 and Nanog transcription network regulates pluripotency in mouse embryonic stem cells. *Nat. Genet.* 2006, 38:431-440.
36. Maas, S., S.E. Tröder, L.K. Ebert, L. Butt, S. Assenmacher, B. Schermer, et al. An optimized electroporation approach for efficient CRISPR/Cas9 genome editing in murine zygotes. *PLoS One* 2018, 13:
37. Macfarlan, T.S., W.D. Gifford, S. Driscoll, K. Lettieri, H.M. Rowe, D. Bonanomi, et al. Embryonic stem cell potency fluctuates with endogenous retrovirus activity. *Nature* 2012, 487:57-63.
38. Mager, D.L., and J.P. Stoye. Mammalian Endogenous Retroviruses. *Microbiology spectrum* 2015, 3:Mdna3-0009-2014.
39. Maksakova, I.A., P.J. Thompson, P. Goyal, S.J. Jones, P.B. Singh, M.M. Karimi, et al. Distinct roles of KAP1, HP1 and G9a/GLP in silencing of the two-cell-specific retrotransposon MERVL in mouse ES cells. *Epigenetics & chromatin* 2013, 6:15.
40. Mehlmann, L.M., T.F. Uliasz, and K.M. Lowther. SNAP23 is required for constitutive and regulated exocytosis in mouse oocytes†. *Biol Reprod* 2019, 101:338-346.
41. Ng, M., T. Fleming, M. Robinson, B. Thomson, N. Graetz, C. Margono, et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 2014, 384:766-781.
42. Ou, X.H., C.C. Zhu, and S.C. Sun. Effects of obesity and diabetes on the epigenetic modification of mammalian gametes. *Journal of cellular physiology* 2019, 234:7847-7855.

43. Rowe, H.M., J. Jakobsson, D. Mesnard, J. Rougemont, S. Reynard, T. Aktas, et al. KAP1 controls endogenous retroviruses in embryonic stem cells. *Nature* 2010, 463:237-240.
44. Schoorlemmer, J., R. Pérez-Palacios, M. Climent, D. Guallar, and P. Muniesa. Regulation of Mouse Retroelement MuERV-L/MERVL Expression by REX1 and Epigenetic Control of Stem Cell Potency. *Frontiers in oncology* 2014, 4:14.
45. Snider, A.P., and J.R. Wood. Obesity induces ovarian inflammation and reduces oocyte quality. *Reproduction (Cambridge, England)* 2019, 158:R79-r90.
46. Vermeulen, M., H.C. Eberl, F. Matarese, H. Marks, S. Denissov, F. Butter, et al. Quantitative interaction proteomics and genome-wide profiling of epigenetic histone marks and their readers. *Cell* 2010, 142:967-980.
47. Vidacek, N., L. Nanic, S. Ravlic, M. Sopta, M. Geric, G. Gajski, et al. Telomeres, Nutrition, and Longevity: Can We Really Navigate Our Aging? *J. Gerontol. A Biol. Sci. Med. Sci.* 2017, 73:39-47.
48. Volpato, A.M., A. Schultz, E. Magalhães-da-Costa, M.L. Correia, M.B. Águila, and C.A. Mandarim-de-Lacerda. Maternal high-fat diet programs for metabolic disturbances in offspring despite leptin sensitivity. *Neuroendocrinology* 2012, 96:272-284.
49. Wang, T., Y. Cao, Q. Zheng, J. Tu, W. Zhou, J. He, et al. SENP1-Sirt3 Signaling Controls Mitochondrial Protein Acetylation and Metabolism. *Mol. Cell* 2019, 75:823-834 e825.
50. Wu, D., and J. Dean. Maternal factors regulating preimplantation development in mice. *Curr. Top. Dev. Biol.* 2020, 140:317-340.
51. Wu, J., B. Huang, H. Chen, Q. Yin, Y. Liu, Y. Xiang, et al. The landscape of accessible chromatin in mammalian preimplantation embryos. *Nature* 2016, 534:652-657.
52. Xia, W., J. Xu, G. Yu, G. Yao, K. Xu, X. Ma, et al. Resetting histone modifications during human parental-to-zygotic transition. *Science* 2019, 365:353-360.
53. Yamazaki, S., A. Ishii, Y. Kanoh, M. Oda, Y. Nishito, and H. Masai. Rif1 regulates the replication timing domains on the human genome. *The EMBO journal* 2012, 31:3667-3677.
54. Zimmermann, M., F. Lottersberger, S.B. Buonomo, A. Sfeir, and T. de Lange. 53BP1 regulates DSB repair using Rif1 to control 5' end resection. *Science* 2013, 339:700-704.

Figures

Figure 1

Effects of HFD on the metabolic phenotypes in F0 and F1 mice. A, B: Anatomic features of the HFD and CD mice. C: Weekly body weights of F0 mice between the HFD and CD groups. D: Weekly body weights of F1 mice feeding with CD. E: Weekly body weights of F1 mice feeding with HFD. F: Blood glucose levels of F0 mice between the HFD and CD groups when GTTs were applied. G: Blood glucose levels of F0 mice between the HFD and CD groups when ITTs were applied. H: Blood glucose levels of F1 mice between the HFD and CD groups when GTTs were applied. I: Blood glucose levels of F1 mice between the HFD and CD groups when ITTs were applied. *Significant differences compared with the other two groups. HFD: high-fat diet; CD: control diet.

Figure 2

The development potential in oocytes and preimplantation embryos of HFD mice. A: Developmental status captures of oocytes and embryos from the HFD and CD groups. Red asterisks indicated abnormal oocytes and embryos. Bottom: statistical results of developmental status. B: Reactive oxygen species (ROS) of MII oocytes stained by dihydroethidium from the HFD and CD groups. The red fluorescence intensity represented the level of ROS, Bottom: the statistical results were shown between the HFD and CD groups. C: Mitochondrial membrane potential (MMP) of MII oocytes stained by JC-1 from the HFD and CD group. The ratios of the red/green fluorescence intensity indicated the levels of MMP, and Bottom: the statistical results were shown between the HFD and CD groups. D: Representative immunofluorescence results of SIRT3 and PPAR γ in oocytes and blastocysts from the HFD and CD groups. E: Western blot analysis of SIRT3 and PPAR γ in oocytes from the HFD and CD groups. F: qPCR results of SIRT3 and PPAR γ from oocytes to blastocysts between the HFD and CD groups. Throughout, data were presented as means $\pm$ SD. P-value was calculated by Student's t-test. *represents significant differences. n.s., indicates not significant. Scale bars were added in the lower right corner of the captures. HFD: high-fat diet; CD: control diet. BC: blastocyst.

Figure 3

The effects of HFD on RIF1 and epigenetic modification changes in oocytes and preimplantation embryos A: The expressions of RIF1 in oocytes of the HFD and CD groups. Top: representative immunofluorescence (IF) images. Arrowheads indicated the expression of RIF1 located in the nucleus. The bar chart in the middle of bottom: qPCR results of RIF1. The bar chart in the lower right corner: qPCR results of the relative telomere length (RTL). B: Images of γ -H2AX staining in oocytes of the HFD and CD

groups. Bottom: the statistical results were shown. C: The expressions of MuERV-L Gag between the HFD and CD groups. Top: representative IF images in 2-cell embryos. Since MuERVL-Gag could be hardly detected at the other embryonic development stages, only images of 2-cell embryos were presented. Bottom: statistical results from IF and qPCR. D: IF images of 5mC and 5hmC enrichment during ZGA between the HFD and CD groups. The statistical results were shown at the bottom. E: IF images of H3K4me3 and H3K27me3 enrichment from oocyte to blastocyst between the HFD and CD groups. The statistical results were shown at the bottom. Throughout, data were presented as means $\pm$ SD. P-value was calculated by Student's t-test. *represents significant differences. n.s., indicates not significant. Scale bars were added in the lower right corner of the captures. HFD: high-fat diet; CD: control diet. $\boxtimes$ represents maternal pronuclear (PN). $\boxdot$ represents paternal PN.

Figure 4

Metformin alleviated the adverse effects of HFD on oocytes and preimplantation embryos A: Developmental status captures of the oocytes and embryos in the NS-treated and Met-treated groups. Red asterisks indicated abnormal oocytes and embryos. Bottom: statistical results of developmental status. B: Reactive oxygen species (ROS) of MII oocytes stained by dihydroethidium in the NS-treated and Met-treated groups. The red fluorescence intensity represented the level of ROS, Bottom: the statistical results were shown. C: Mitochondrial membrane potential (MMP) of MII oocytes stained by JC-1 in the

NS-treated and Met-treated groups. The ratios of the red/green fluorescence intensity indicated the levels of MMP, and Bottom: the statistical results were shown. D: Images of γ -H2AX staining in oocytes of the NS-treated and Met-treated groups. Bottom, the statistical results were shown. E: The expressions of RIF1 in oocytes of the NS-treated and Met-treated groups. Top: representative IF images. Middle: statistical analysis results from IF. Bottom: qPCR results of the relative telomere length (RTL) of the NS-treated and Met-treated oocyte. F: The expressions of MuERV-L Gag in the 2-cell embryos of the NS-treated and Met-treated groups. G: IF images of 5mC and 5hmC enrichment during ZGA between the NS-treated and Met-treated groups. The statistical results were shown at the bottom. H: IF images of H3K4me3 and H3K27me3 enrichment during ZGA between the NS-treated and Met-treated groups. Throughout, data were presented as means $\pm$ SD. P-value was calculated by Student's t-test. *represents significant differences. n.s., indicates not significant. Scale bars were added in the lower right corner of the captures. NS-treated: normal saline-treated; Met-treated: metformin-treated. $\boxtimes$ represents maternal pronuclear (PN). $\boxdot$ represents paternal PN.

Figure 5

RIF1 regulates MuERV-L interacting with H3K4me3 and H3k9me3. A: Validation of the effectiveness of electroporation, and experiments of various concentrations of mCherry-Trim21 mRNA and RIF1 antibody. Top: various concentrations of mCherry-Trim21 mRNA were evaluated, proving the effectiveness of

electroporation. Bottom: various concentrations of RIF1 antibody were evaluated with the developmental abilities of embryos. B: The remaining protein intensities of endogenous RIF1 were obtained between the RIF1 KD and control groups when the 10 ng/μl RIF1 antibody was electroporated into the oocytes. Bottom: statistical result was shown. C: The expressions of MuERV-L in the 2-cell embryos of the RIF1 KD and control groups. Top: representative immunofluorescence (IF) images of 2-cell embryos. Bottom: statistical results from IF and qPCR. D: IF images of 5mC and 5hmC enrichment during ZGA between the RIF1 KD and control groups. The statistical results were shown at the bottom. E: IF images of H3K4me3 and H3K27me3 enrichment during ZGA between the RIF1 KD and control groups. F. ChIP-seq track signals of H3K4me3, H3K9me3 and H3K27me3 from mouse oocyte to blastocyst, which were extracted from GSE73952 and GSE 98149, and visualized by Integrative Genomics Viewer (IGV). G: Track signals of mRNA expression, Rif1, H3K9me3, and H3K27me3 ChIP-seq and ATAC-seq signals at the MERVL-int locus in Control and RIF1 KD ESCs, which were extracted from GSE98356. Throughout, data are presented as means ± SD. P-value was calculated by Student's t-test. *represents significant differences. n.s., indicates not significant. Scale bars were added in the lower right corner of the captures. RIF1 KD: RIF1 knockdown. ♂: represents maternal pronuclear (PN). ♀ represents paternal PN.

Figure 6

Diagram summarizes the influencing factors of offspring phenotype and the proposed epigenetic mechanism in a HFD mice model.

Supplementary Files

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