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Experimental Research Article

Histone methyltransferase G9a inhibitor (UNC0631) reinforces mitochondrial function and upregulates UCP1 in brown adipocytes and screening of epigenetic libraries

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ABSTRACT

Introduction: Obesity leads to massive death worldwide by initiating numerous illnesses like Nonalcoholic Steatohepatitis (NASH), liver disease, and cardiovascular diseases. Developing new therapeutics against obesity is an emergency need. Targeting mitochondrial uncoupling protein 1 (UCP1) will provide new therapeutic strategies for drug discovery research against obesity and obesity-related disorders.

Methods: We screened UCP1 up-regulators from epigenetic drug libraries by using a previously developed Ucp1-A-GFP cellular GFP screening platform, ATP production, and mitochondrial DNA quantification.

Results: We discovered that the histone methyltransferase G9a inhibitor UNC0631 has a considerable effect on the expression of UCP1 in adipocytes when used in vitro. Here, we discovered that UNC0631 is crucial for controlling mitochondrial activity and anti-obesity. The UNC0631-treated fat cells have higher UCP1 expression at the cellular level. Taken together, in our studies, we have established an efficient in vitro cell experiment system to study the metabolic regulation of UCP1. Enhanced mitochondrial DNA, ATP synthesis, and cell survival showed that UNC0631 had a benign impact on the HEK293T cell line. As a result, UNC0631 reveals a promising therapeutic option for the treatment of diseases associated with obesity and metabolic disorders.

Conclusion: In this study, we make a list of potent drug candidates from epigenetic drug libraries that can upregulate mitochondrial UCP1 gene expression and promote thermogenesis. UNC0631 improves mitochondrial function and would be an effective drug candidate to treat metabolic diseases and obesity-related diseases. Further investigation will require both the human and animal models to reveal new insight into the mechanism against obesity, metabolic diseases, or mitochondrial dysfunction-related diseases.

Keywords:
Obesity
UCP1
Histone methyltransferase
Mitochondrial function
Epigenetic drug libraries
Metabolic disorders

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Introduction

Obesity is the main precursor to developing several illnesses worldwide. Developing new therapeutics against obesity is an emergency need. Overweight and obese adults are predicted to make up about 57.8% (3.3 billion) of the adult populace worldwide by 2030 (Chang et al., 2019; Chouchani et al., 2019; Reyad-ul-Ferdous et al., 2022b). Numerous illnesses, such as liver disease, cancer, diabetes, and cardiovascular disease, could be brought on by the global obesity epidemic. Developing new therapeutics against obesity is an emergency need (Ferdous et al., 2022; Kose et al., 2015; Li et al., 2022; Perrio et al., 2007).

The physiological implications of these changes are very convincing. Mice exhibit enhanced resistance to obesity gain after high-fat feeding and total body energy utilization. Adipocytes show significantly elevated rates of mitochondrial respiration. Additionally, exposure of mouse brown adipose tissue (BAT) BAT to thermal stress (40°C) or adrenergic stimulation leads to an increase in lipid hydroperoxides, mitochondrial hydrogen peroxide, and mitochondrial superoxides (Chouchani et al., 2016; Mailloux et al., 2012; Reyad-ul-ferdous and Azam 2020; Stier et al., 2014).

The UCP1 tightly controls proton grade, which is necessary for the production of ATP. UCP1 activates the sympathetic nervous system and activates adrenergic receptors in response to cold. It also induces thermogenesis in adipocytes (Nicholls 2006; Saito et al., 2009).

The proximal region of the UCP1 promoter was found to interact with the cold-inducible zinc finger protein 516 (Zfp516) (Dempersmier et al., 2015; Sambeat et al., 2016). The expression of UCP1 and Pgc-1 can also be induced by Prdm16 and Zfp516 complexes. Zfp516 deficiency has resulted in embryonic mortality and a significant decrease in BAT mass. However, by increasing energy expenditure, diet-induced obesity and thermogenic adipocytes can be initiated in WAT depots can be prevented (Sambeat et al., 2016). Adenosine and ribonucleosides play a crucial role in signal transduction and energy transfer, which regulate lipolysis and respiration in mouse and hamster models. Furthermore, activation of adenosine receptors helps prevent diet-induced obesity by enhancing energy expenditure (Gnad et al., 2014; Reyad-ul-Ferdous et al., 2022a).

Finding viable pharmacological options to upregulate UCP1 in brown and white adipocytes was the main goal of the experimental work. Due to the limited availability of effective medications for metabolic disorders and obesity, the mortality associated with these conditions has significantly increased over the past few decades. Therefore, there is an urgent need to identify novel drug candidates to treat obesity-related disorders.

Previous research suggests that several medications have the ability to increase the expression of the UCP1 gene, however, these medications also have several negative side effects and can cause mild to severe illnesses or symptoms. Previous research showed that UNC0631 has great cellular potency and excellent separation of functional potency versus cell toxicity in a range of cell lines. UNC0631 has negligible cell toxicity and is quite effective at lowering H3K9me2 levels.

Recent research has focused on brown adipocytes governing to improve energy utilization and provide an effective new strategy for treating obesity and metabolic disorders (Betz and Enerback 2015; Harms and Seale 2013; Reyad-ul-ferdous and Azam 2020; Rosen and Spiegelman 2014). Particularly, the discovery of active brown adipocytes in the human body has spurred interest in increasing energy expenditure as a potential treatment for metabolic disorders and related diseases (Azam et al., 2022; Cypess et al., 2009; Reyad-ul-Ferdous M 2014; Saito et al., 2009; ul Ferdous 2014; Virtanen et al., 2009). Our goal is to identify effective epigenetic drugs for the treatment of metabolic disorders or obesity by upregulating UCP1 in adipocytes. These drugs will provide a successful and safe therapeutic regimen.

In this study, we intended to provide a list of epigenetic drugs that can unregulate mitochondrial UCP1 gene expression in brown adipocytes. Further study will uncover new insight into the mechanism of action of epigenetic drug candidates against obesity or mitochondrial function-related metabolic diseases.

Material and methods

Antibodies, Chemicals, and Reagents

The selleckchem was used to acquire the Epigenetic medicines library ((96-well)-Z234081-100ul-L1900-01-03) and 187 inhibitors. As mentioned, additional reagents were procured:

UNC0631 (Selleck, S7610), T3 (Sigma, T2877), Dexamethasone (Sigma, D4902), insulin (Sigma, I3536), indomethacin (Sigma, I7378), isobutylmethylxanthine, rosiglitazone (Santa Cruz, sc202795) are

examples of medications (Sigma, I5879). HSP90 (Cell Signaling Technology, 4874S), β-Actin (Abclonal, AC004), and UCP1 (Abcam, ab10983). Fetal bovine serum (FBS), 3-isobutyl-1-methylxanthine (IBMX), rosiglitazone (Rosi), and high-glucose, A streptomycin-penicillin solution were bought from Hyclone Laboratories, Inc., and Dulbecco's modified Eagle's medium (DMEM) was purchased from Atlas Biologicals (Fort Collins, CO, USA) (South Logan, NY, USA). Compound CID: 53315868, MW: 635.9g/mol, MF: C37H-61N7O2; UNC-0631; CHEMBL1829305.

IUPAC Name: N-[1-(cyclohexylmethyl)piperidin-4-yl]-6-methoxy-7-(3-piperidin-1-ylpropoxy)-2-(4-propan-2-yl-1,4-diazepan-1-yl)quinazolin-4-amine.

Cell Treatment Protocol

Pre-adipocytes (white and brown) were grown in high-glucose Dulbecco's modified Eagle's medium (DMEM) with penicillin/streptomycin at a 1% concentration and fetal bovine serum (FBS) at a 20% concentration and fetal bovine serum (FBS) at a 20% concentration. The cells have been consistently used in all studies from day 6 of the differentiation process, except for those that were newly initiated (Ferdous et al., 2022; Qiu et al., 2018; Reyad-ul-Ferdous et al., 2022a).

Confluent cells were designated as day 0, and the differentiation process was initiated using an induction medium for three days, as detailed in Supplementary Table 1. Following this period, the daily maintenance medium was modified, as described in the same table. The culture of white pre-adipocytes followed the same steps as the differentiation of brown pre-adipocytes, except that the maintenance and induction media contained an insulin concentration of 5 g/mL (Qiu et al., 2018; Reyad-ul-Ferdous et al., 2022a; Reyad-Ul-Ferdous and Song 2022). To uncover new, distinct drug candidates, cells were treated either throughout the differentiation phases or after day 8 of differentiation (Supplementary Figure 1).

Compounds or Chemicals Identification

Brown and white pre-adipocytes of the UCP1-2A-GFP reported adipocyte cell line were planted in 96-well plates, where they underwent differentiation and were subjected to drug testing. The cells were treated on day 8 of the differentiation process with specific chemicals at a concentration of 10 mM, and on days 8 through 13, the outcomes were determined by visually monitoring the GFP intensity. To identify target candidates, the natural drug libraries were purchased from Selleck (L1400). Supplementary Figure 1 lists the cells that were treated with DMSO as a negative control and isoproterenol (ISO) as a positive control.

Western blotting

Protein extraction from brown pre-adipocyte cells was performed using RIPA buffer (Millipore, 20188), followed by western blot analysis. Protein lysates were prepared using radioimmunoprecipitation assay (RIPA) buffer (Millipore, 20,188) containing 50 mmol/L Tris, pH 7.4, 1% NP40, 0.25% sodium deoxycholate, 150 mmol/L NaCl, 1 mmol/L EDTA, 1 mmol/L phenylmethylsulfonyl fluoride, and protease inhibitor cocktail (Roche, Lewes, United Kingdom). The lysates were stored at −80°C for 30 minutes, then thawed on ice and centrifuged at 12,000 rpm for 10 minutes at 4°C. The supernatant was collected, and total protein concentration was determined using a Bio-Rad assay (Bio-Rad Laboratories). Anti-mouse and anti-rabbit secondary antibodies (Dako), conjugated with BSA, were added at a dilution of 1:1,000. Equal protein loading was confirmed using HSP90 as a loading control. Bands were visualized with the ECL detection system (GE Healthcare, United Kingdom), and autoradiographic films were scanned. Band intensities were quantified using ImageJ densitometry (https://imagej.nih.gov/ij/) and normalized to *HSP90 levels* (Ferdous et al., 2022; Reyad-ul-Ferdous et al., 2022a). A list of primary antibodies used in the experiment is provided in Supplementary Table 1.

ATP Production

The rate of ATP synthesis in both live white and brown pre-adipocytes was measured using the Luminescent ATP Detection Assay Kit (Abcam, ab113849), following the manufacturer's instructions (Ferdous et al., 2022; Reyad-ul-Ferdous et al., 2022a).

Mitochondrial DNA Quantification

Genomic DNA was extracted from brown and white adipocyte-treated cells using the TIANamp Genomic DNA Kit (Tiangen, DP304-03). The nucleic acid concentration in the samples was measured using a NanoDrop spectrophotometer (Thermo Scientific), and the volume was normalized to 100 ng/µL. The NovaQUANT Mouse

Mitochondrial to Nuclear DNA Ratio Kit (catalog no. 72621; Merck) was used to quantify mitochondrial DNA (mtDNA) relative to nuclear DNA (nuDNA). RT-PCR was used to determine the nuDNA to mtDNA ratio (Reyad-ul-Ferdous et al., 2022a) by amplifying both mtDNA and nuDNA using qPCR. The following primers were used: For mtDNA, 5'-CCTATCACCCTTGCCATCAT, and 5'-GAGGCTGTTGCTTGTGAC, while for nuDNA, 5'-DNAATGGAAAGCCTGCCATCATG-3' and 5'-TCCTTGTTGTTCAGCATCAC-3'. The mtDNA/nuDNA ratio was determined using the ΔΔCT technique (Ferdous et al., 2022; Reyad-ul-Ferdous et al., 2022b; Reyad-Ul-Ferdous and Song 2022).

Cell Viability Assay by CCK-8 Test

To perform an in-vitro cytotoxic assay, the human embryonic kidney (HEK293T) cells were seeded at a density of 1.3×10^5 cells per well in 96-well plates. Cytotoxicity was assessed using the Cell Counting Kit-8 (Dojindo Laboratories, Tokyo, Japan). Epi H6 (UNC0631) concentrations for 24- and 48-hour treatments were 0, 1, 5, and 10 mg/mL. The cell viability experiment was carried out according to the manufacturer's instructions (Bao et al., 2019; Mu et al., 2018; Reyad-ul-Ferdous et al., 2022a; Yin et al., 2019).

Ethics approval

This study was approved by the university ethical board of Shandong provincial hospital, Shandong University. No animal or human samples were used in this study.

Statistics

The statistical analysis was carried out using Graph Pad Prism 9 (Graph Pad Software Inc.) and SPSS (SPSS 18.0; IBM Japan). Data are presented as means ± standard error of the mean (SEM). For two-group comparisons, an appropriate unpaired Student's t-test has been performed. Multiple group comparisons were conducted using one-way or two-way ANOVA, followed by post hoc Tukey's test, as appropriate. The level of statistical significance was determined based on P-values: ****p0.0001, ***p 0.001, **p 0.01, and *p 0.05.

Results

Epigenetic Drug Library screening

We chose an epigenetic drug library to identify potent therapeutic candidates that can increase the expression of the UCP1 gene. We conducted a screening of the 181 compounds (various types of inhibitors) in the epigenetic drug library. The epigenetic medications displayed significant pharmacological properties by controlling several processes, including histone acetylation, DNA methylation, histone demethylases, and histone deacetylation. Environmental cues as well as stimuli, such as hormonal or nutritional state, cold temperature, and environmental signals, regulate the activity and expression of epigenetic effectors. In adipocyte tissues, chromatin-modifying enzymes that either add (writers) or eliminate (erasers) epigenetic effects on lysine residues of histone H3, such as histone methylation or acetylation, affect the expression of the thermogenic genes as well as metabolic enzymes. The thermogenic adipose program, which aids in adipocytes' ability to maintain homeostasis of energy, can therefore be activated (+) or suppressed (-) in response to various external stimuli through epigenetic regulation. In a feedback loop, intracellular metabolic pathways regulate thermogenesis by modulating the availability of unsaturated fats and glucose, as well as influencing epigenetic processes through molecules such acetyl-CoA, NAD+, and FAD. While confirming their biological significance for the thermogenic lipid program, it will take a lot of work to define epigenetic molecular pathways and their exact targets (Sambeat et al., 2017). We identified a novel candidate epigenetic drug that increases UCP1 gene expression in brown adipocytes, as described in Supplementary Table 2 and Supplementary Table 3, with chemical structures listed in Supplementary Table 3. The elevation of UCP1 gene expression is indicated by the color green.

Epi H6 (UNC0631) Promotes iBAT Adipocyte UCP1 Expression

Through individual studies of the epigenetic drug library, we validated some of the screening results. We found that the GFP intensity increased significantly with each of the tested drugs candidates. Treatment with the adrenergic agonist isoproterenol (ISO), which is consistent with the screening results, resulted in increased GFP intensity among all the agonists studied (Figure 1A). We then focused on Epi H6 (UNC0631), which had the highest ratio of relative GFP intensity among the undisclosed medicines Epi H6 (UNC0631).

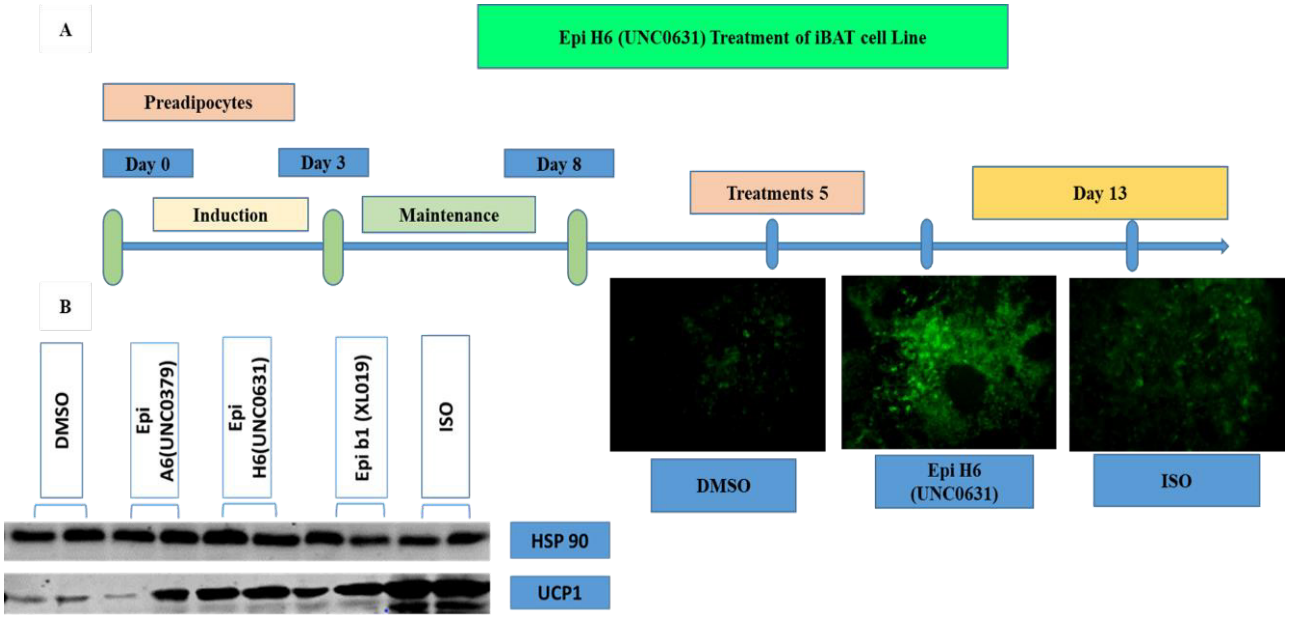

FIGURE 1. UCP1 gene expression in iBAT cell lines is significantly increased by the epigenetic drug library Epi H6 (UNC0631). (A) Schematic diagram showing GFP expression in iBAT adipocytes after 5 days of Epi H6 (UNC0631) treatment (Treatment began after 8 days). (B) Western blot analysis of UCP1 protein expression in Ucp1-2A-GFP brown adipocytes treated for 5 days with Epi H6 (UNC0631), isoproterenol (ISO), or vehicle (DMSO). (n=3 per treatment group).

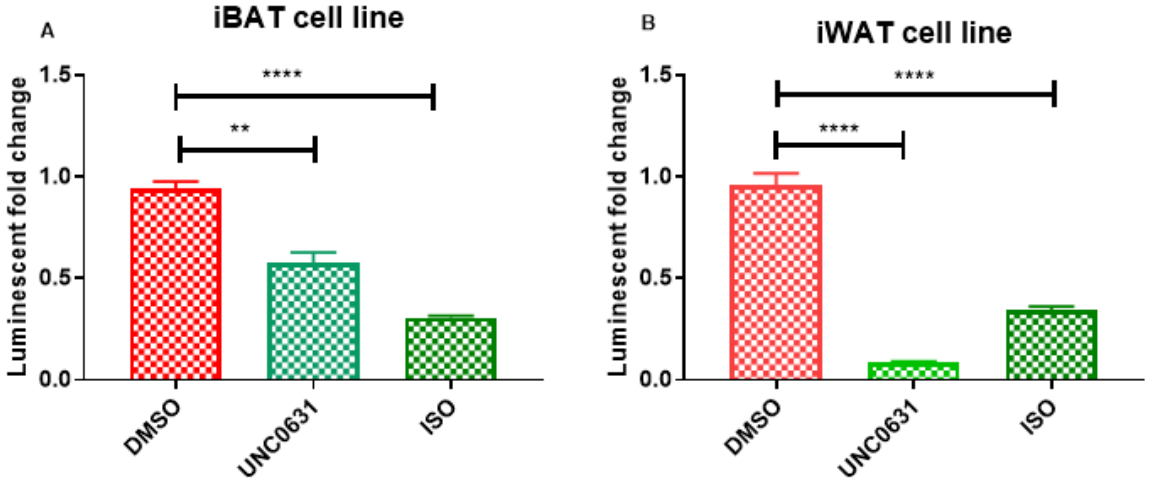

FIGURE 2. Effect of mitochondrial ATP production by UNC0631 epigenetic therapeutic candidates on iBAT and iWATcell lines.(A) Brown adipocyte cell line treated with drug after differentiated cell maturation. (B) White adipocyte cell line treated with drug after differentiated cell maturation. (n=3 per treatment group). Data are presented as means ± SEM. (Unpaired, two-tailed Student's t-test) $p< 0.05$, $p< 0.01$, $p< 0.001$, and $p< 0.0001$.

Protein lysine methyltransferase G9a is crucial for the transcriptional repression of many different genes by methylating lysine 9 on histone H3 (H3K9me2) of chromatin and non-histone proteins, such as the tumor suppressor p53. With an IC50 of 4 nM, UNC0631 is a powerful inhibitor of histone methyltransferase G9a (Zhao et al., 2019).

We initially investigated the effects of Epi H6 (UNC0631) on in vitro cultivated brown and white adipocytes. The results showed that treatment with Epi H6 significantly increased the UCP1 GFP signal and UCP1 protein expression levels in brown adipocytes, as demonstrated in Figures 1A and 1B.

Epigenetic Drug (UNC0631) ATP Production Using iWAT and iBAT Cell Lines

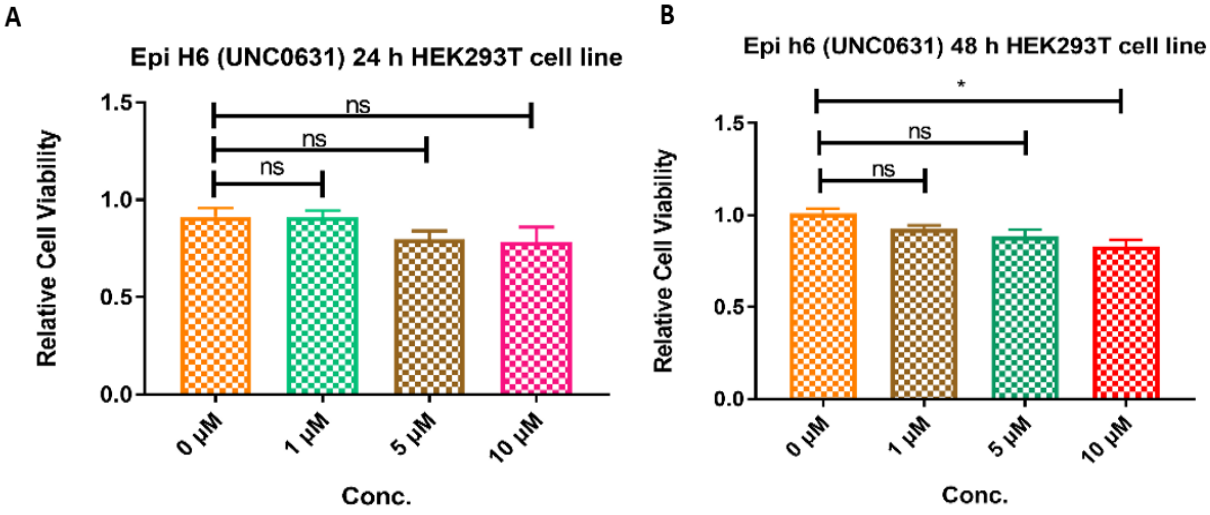

FIGURE 3. Cytotoxic effects of epigenetic therapeutic candidates (UNC0631) on HEK 293T cell lines. Treatment with an increased UCP1 gene expression, an epigenetic therapeutic candidate selected from a library, or a control (n=3 per treatment group). Data are presented as means ± SEM. *p<0.05, **p<0.01, ****p<0.001, ****p<0.0001 (unpaired, two-tailed Student's t-test).

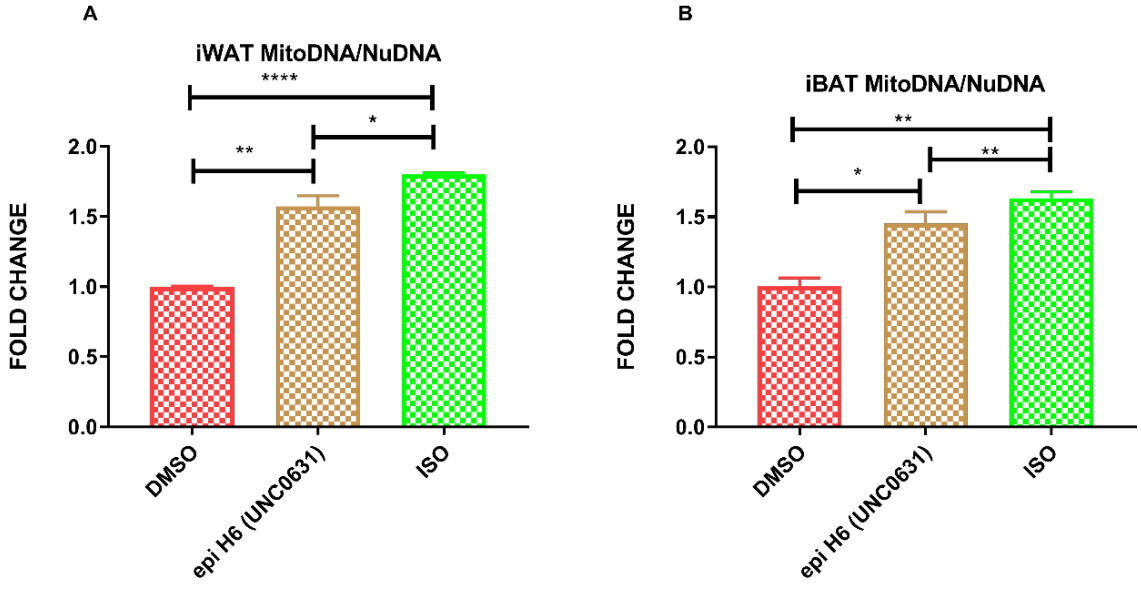

FIGURE 4. Relative quantification of mitochondrial DNA in iWAT and iBAT cell lines. Epi H6 (UNC0631) or the control (vehicle) was administered to iWAT and iBAT cell lines (n = 3 per treatment group). Data are presented as means ± SEM. Data are presented as means ± SEM. *p<0.05, **p<0.01, ****p<0.001, ****p<0.0001 (unpaired, two-tailed Student's t-test).

Upon administering different drugs identified through epigenetic library screening to iBAT cells, ATP generation decreased in comparison to the DMSO-treated cell group (Epi H6 (UNC063) (-0.39)fold change), which is in line with the increased UCP1 expression displayed in Figures 2A–E. This shows that the activation of the UCP1 gene prolonged the energy uncoupling process because the treated epigenetic drug library cells generated ATP at a lower level than the control DMSO-treated cells, despite an enhanced in mitochondrial activity. The reduced ATP generation of other drugs screened from an epigenetic library treated in iWAT cells (Epi H6 (UNC063) (-0.39) fold change in treatment group, respectively, is similar with the increased UCP1 expression seen in Figure 2 A–E. In contrast to the vehicle DMSO group shown in Figure 2 C, Epi A6 does not significantly improve ATP generation. Epi A6 (UNC0379) may be responsible for upregulating ATP synthesis in

iWAT adipocytes. Despite the elevated levels of mitochondrial activity, the epigenetic medicines library treated cells group dramatically reduced its ATP generation as compared to the vehicle DMSO group, indicating enhanced energy uncoupling through upregulated UCP1 gene expression.

Epigenetic Drugs (UNC0631) Restrain Cell SurvivalUsing HEK293T Cell Lines

Additionally, we examine the toxicity of several selected drug candidates using functional cell survival tests, epigenetic drug libraries, and increased UCP1 expression. For the selected drug candidates, 48 hours of treatment with Epi h6 (UNC0631) on the HEK293T cell line indicates considerable cell death (0.18-fold change) at the exclusive drug 10 mM concentration, as shown in Figure 3 C.

Mitochondrial Copy Number(Treatment with epi H6 (UNC0631) using adipocytes cells)

The histone methyltransferase G9a inhibitor UNC0631 is a unique, significant, and specific compound. Protein lysine methyltransferase G9a plays important functions in the transcriptional regulation of numerous genes by demethylating histone H3 (H3K9me2) and non-histone proteins, such as the tumor suppressor p53, at lysine 9 in the chromatin. In contrast to the vehicle DMSO group, the iBAT cell line treated with epi H6 (UNC0631) (1.49-fold change) and the positive control ISO (1.67-fold change) considerably extended. However, in contrast to the vehicle DMSO group shown in Figures 4 A and B, the iWAT cell line treated with epi H6 (UNC0631) (1.67-fold change) and positive control ISO (1.75-fold change) both considerably increased. This finding demonstrated that epi H6 (UNC0631) administration increases mitochondrial activity in both brown and white adipocytes by upregulating the expression of the UCP1 gene. According to our findings, lysine methyltransferase G9a is regulated by epi H6 (UNC0631), which in turn modulates mitochondrial function. In addition to its other potential functions, Epi H6 (UNC0631) may also play a crucial part in the epigenetic regulation of the thermogenesis pathway through UCP1 gene expression. However, further research is needed to elucidate the mechanisms by which mitochondrial uncoupling protein-1 is modified and regulated.

Discussion

The accurate progression of adipocyte and osteoblast differentiation is controlled by several co-regulators, which are important promoters of the histone acetylation, methylation, DNA methylation, and nucleosome rearrangement processes. This study looked at the mRNA expression of the H3K9 demethylase, KDM4A, in a variety of mouse tissues and found that bone and skeletal muscle had higher levels of KDM4A expression than other tissues. Additionally, KDM4A exhibited higher expression levels throughout the development of mouse stromal cells, whether during adipogenic or osteogenic differentiation (Qi et al., 2019; Reyad-ul-Ferdous et al., 2022b). Histone methyl-modifiers (EZH2) block polycomb repressive complex 2 (PRC2), an enzyme subunit that catalyzes the trimethylation of histone H3K27me3, and this, in turn, encourages beige adipocyte synthesis in diet-induced obese mice (Wu et al., 2018). Through its demethylase activity, the histone H3K9me1/me2 demethylase JMJD1A causes beige adipogenesis. By demethylating H3K9me2, JMJD1A is recruited to the regions of specific genes in response to cold by being phosphorylated at S265 and inducing its recruitment to these areas. This results in persistent beige adipogenesis. Phosphorylated JMJD1A in brown adipocytes rapidly changes the structure of the chromatin and activates the expression of heat-generating genes via an enzyme-independent process (Abe et al., 2018; Abe et al., 2015).

In this study, we screened an epigenetic compound library comprising various inhibitors targeting DNA/RNA synthesis, HDAC, JAK, Pim, DNA methyltransferase, FLT3, epigenetic reader domains, and histone methyltransferases. The primary aim was to identify potential epigenetic drug candidates capable of regulating UCP1 expression. The H3K27 histone demethylase JMJD3 was thought to be strongly inhibited by GSK J4 HCl, and UTX, which could dramatically boost UCP1 expression and activate thermogenic genes in brown adipocytes in-vitro cell model, was also successfully discovered as a sort of modifier inhibitor (Ferdous et al., 2022; Reyad-ul-Ferdous et al., 2022b). Recent research has highlighted that histone methylation is precisely regulated by histone methyltransferases and demethylases. These enzymes are also involved in cellular processes like cell differentiation, apoptosis, and the progression and development of various diseases (Ferdous et al., 2022; Kelly and Issa 2017; Reyad-ul-Ferdous et

al., 2022a; Reyad-ul-Ferdous et al., 2022b).

By maintaining the energy level, regulating several metabolic activities, and controlling apoptosis, mitochondria play a crucial role in maintaining cell homeostasis. So, cellular stress is undoubtedly mostly caused by mitochondrial malfunction. Additionally, numerous human diseases like diabetes, cancer, and neurological disorders have been linked to energy changes. One hypothesized characteristic of tumor cells, particularly in cancer, is the dysregulation of cellular energetics (Hanahan and Weinberg 2011; Kwak et al., 2010). Furthermore, it is not surprising that mitochondrial metabolites can regulate host nucleus gene expression under stressful circumstances characterized by an unchecked pace of proliferation. Transcriptome changes have been discussed concerning the malfunctioning of the mitochondria in mammalian tissues and cells (Reyad-ul-Ferdous et al., 2022b; Zhang and Falk 2014). Epigenetic changes have the ability to control gene expression quickly, dynamically, and irreversibly. MicroRNAs, DNA methylation, and histone modifications all control how genes are expressed by modifying the chromatin structure or preventing protein translation. It has been suggested that there may be a connection between the altered epigenome and mitochondrial function (Afanas'ev 2014; Peng et al., 2011; Reyad-ul-Ferdous et al., 2022b; Zhou et al., 2016). Since important epigenetic factors are modified by mitochondrial metabolites (Hanahan and Weinberg 2011; Kreuz and Fischle 2016; Kwak et al., 2010; Reyad-ul-Ferdous et al., 2022b).

We identified epigenetic medication libraries that might be dramatically repurposed by upregulating UCP1 gene expression to treat obesity using previously established screening platforms from our lab. As a result, we confirmed several medications that can increase the expression of UCP1 in brown adipocytes, proving the viability of using this method to choose UCP1 activators. Additional research focuses on a novel drug library that includes the previously undisclosed medication UNC0631 from the epigenetic drug library.

In the current work, we examined the common characteristics associated with mitochondrial (UCP1) gene expression as shown in Figures 1A–C to determine the effectiveness of the epigenetic medication UNC0631 in a cellular model. The toxic effect of UNC0631 is then examined, with different concentrations having a non-toxic effect on the HEK 293 T cell line. No harmful effect was noticed with repeated use in mice, as shown in Figures 3A, and B. Our findings showed that UNC0631 affects mitochondrial activity by lowering the level of ATP synthesis seen in Figures 2A and 2B. By increasing the expression of the UCP1 gene, decreased mitochondrial ATP synthesis increases thermogenesis. The growing attention given to brown adipocytes has stoked interest in their potential benefits for treating metabolic illnesses. Uncoupling protein 1 (UCP1) is expressed at increased levels in brown and beige adipocytes in place of ATP synthesis to dissipate heat. Enlisting UCP1 increases nonshivering thermogenesis, resulting in increased energy expenditure and obesity inhibition. This is accomplished through increasing nonshivering thermogenesis by cold, exercise, and food. The existence of utilitarian UCP1-independent thermogenic controls has recently been shown in adipocyte studies. In addition, in the absence of UCP1, white adipocytes can increase energy expenditure through substrate cycling including cold-induced N-acyl amino acids, creatine metabolites, and oxidized lipids. These studies highlight the importance of taking into account the mechanisms underlying adipocytes' ongoing energy expenditure as well as their upcoming demands in the prevention of obesity and metabolic disorders in humans (Jastroch et al., 2010; Jonckheere et al., 2012; Quintana-Cabrera et al., 2018; Reyad-ul-Ferdous et al., 2022a; Young et al., 2017).

Overall, the findings showed that brown adipocytes can express more UCP1, have more mitochondria, and are more active when treated with UNC0631. UNC0631 demonstrated anti-proliferative at lower drug concentrations in malignant cells. Post-translational epigenetic modification depends on histone methylation. Recent research has shown that two enzymes, histone methyltransferases, and demethylases, are precisely responsible for controlling the methylation of histones. These enzymes are also involved in cellular processes like cell differentiation and apoptosis, as well as the progression and development of many diseases. In multicellular organisms, the epigenetic state of cells is critical in establishing their differentiation status as well as their appropriate function. The in vivo mechanism of action of the epigenetic medicines found using this screening technology has to be clarified by an animal investigation. Through the direct or indirect elevation of UCP1 gene expression, UNC0631 and the medications listed in supplementary Table 2 and supplementary Table 3

demonstrated potential pharmacological benefits, including lipid-lowering effects, antidiabetic activity, and enhanced energy expenditure. Our research identified several promising epigenetic medication candidates that could be more effective and safer for treating metabolic disorders associated with UCP1 dysfunction and obesity-related diseases. Further research is needed to analyze their RNA-seq, metabolomic, and CMAP results.

Conclusion

The availability of medications for treating obesity is currently very limited, with many drugs frequently being withdrawn from the market due to serious side effects. Although inducing browning in white adipose tissue (WAT) is a promising approach for addressing obesity and metabolic disorders, more research in humans is needed to determine the effectiveness of these agents in sustaining a browning response and to assess potential long-term adverse effects. Modern research has focused on elucidating the roles of different types of adipocytes, including WAT and iBAT. Adipocyte promotes the progression of heat in response to clod expression besides burning calories by utilizing fatty acid as well as glucose. Since the identification of human functional iBAT adipocytes, targeting iBAT adipocytes has been a promising therapeutic strategy for controlling metabolic disorders. In conclusion, we believe that our research advances the ongoing endeavor to identify pharmacological candidate moieties that stimulate adipose tissue UCP1 gene expression. Specifically, we introduce a list of epigenetic drug libraries that may be used to treat obesity or metabolic illnesses associated with obesity as well as an easy-to-use cellular screening technique for UCP1 activators to conduct screenings on this platform for potentially millions of drug moieties. More in-depth research into these drugs and their underlying mechanisms may provide new insights into the mechanistic route in the adipose role and help to build medicines against obesity and other metabolic diseases. Our research also reveals several effective therapeutic candidates from the epigenetic drug library that can greatly increase the expression of the UCP1 gene and improve mitochondrial functioning. The in-vivo mechanistic pathway with a variety of targets in the murine model has to be clarified through additional research. However, this study contributes to existing obesity or metabolic-related diseases in several ways. First, In the current conditions, no more effective drug candidates are available against obesity. Second, a limited number of drugs are available for the treatment of obesity but prolonged use promotes several severe side effects, for example, Atorvastatin; an effective lipid-lowering drug causes severe side effects including urinary tract infection, and liver function test abnormal, AST increased, and so on. Our investigation identifies of new drug candidate which can upregulate UCP1 expression and significantly reduces body weight. Which provokes advancements against metabolic disorders or obesity. Obesity, cardiovascular disease, cancer, and type-2 diabetes are among the illnesses associated with white adipocyte and brown adipocyte malfunction. Finding novel medicines is a demand that has emerged in this situation. In order to significantly increase the expression of mitochondrial UCP1 and control adipocyte dysfunction, we create candidate medication lists from epigenetic drug libraries. With the help of epigenetic drug libraries, we were able to isolate a number of unique target-binding compounds that have the potential to shed new light on the pharmacology and mechanism of action of many metabolic disorders and other diseases that are closely related to them. Here, we evaluate the functional roles of UNC0631 that edivant nontoxic, effective to regulate mitochondrial function by upregulating mitochondrial DNA copy number and ATP systhesis (thermogenesis). Further investigations requires to potential mechanistic pathway from human samples and clinical trails.

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