Inhibitory effects of high stability fucoxanthin on palmitic acid-induced lipid accumulation in human adipose-derived stem cells through modulation of long non-coding RNA

Journal:	*Food & Function*
Manuscript ID:	FO-ART-03-2015-000301.R2
Article Type:	Paper
Date Submitted by the Author:	09-May-2015

Complete List of Authors:	Lin, Wen-Chuan; Shuang-Ho Hospital, Taipei Medical University, Department of Pediatrics Shih, Ping-Hsiao; Shuang-Ho Hospital, Taipei Medical University, Department of Pediatrics Wang, Weu; Taipei Medical University Hospital,Taipei Medical University, Comprehensive Weight Management Centre Wu, Chi-Hao; College of Public Health and Nutrition,Taipei Medical University, School of Nutrition and Health Sciences Hsia, Shih-Min; College of Public Health and Nutrition,Taipei Medical University, School of Nutrition and Health Sciences Wang, Hsian-Jenn; Wan-Fang Hospital, Taipei Medical University, Department of Plastic Surgery Hwang, Pei-An; Fisheries Research Institute, Council of Agriculture,Keelung, Seafood Technology Division Wang, Chuan-Yu; Shuang-Ho Hospital, Taipei Medical University, Department of Pediatrics Chen, Shu-Huey; Shuang-Ho Hospital, Taipei Medical University, Department of Pediatrics Kuo, Yung-Ting; Shuang-Ho Hospital, Taipei Medical University, Department of Pediatrics

Food & Function

RSCPublishing

ARTICLE

Inhibitory effects of high stability fucoxanthin on palmitic acid-induced lipid accumulation in human adipose-derived stem cells through modulation of long non-coding RNA

Cite this: DOI: 10.1039/x0xx00000x

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

Wen-Chuan Lin, [a$] Ping-Hsiao Shih, [a$] Weu Wang, [b] Chi-Hao Wu, [c] Shih-Min Hsia, [c] Hsian-Jenn Wang [d] Pai-An Hwang, [e] Chuan-Yu Wang, [af] Shu-Huey Chen [af] and Yung-Ting Kuo *[af]

Obesity is a serious and worldwide disease that is growing in epidemic proportions. Adipose-derived stem cell (ADSC) is characterized as a source of mesenchymal stem cell that has been acting as a potential application for regeneration. Recently, seaweeds rich in flavonoids and polysaccharides have been supposed to show the ability to modulate risk factors for obesity and related disease. In the present study, we investigated the anti-obesity properties of high stability fucoxanthin (HS-Fx) derived from brown seaweeds on adipogenesis of ADSC upon the treatment of palmitic acid (PA). First, we showed the differentiation capability of ADSCs from morbid obesity patients to transform into different cell types. We further found that co-treatment of ADSCs with HS-Fx and PA showed no significant cytotoxicity against ADSCs, but PA induced elevation of reactive oxygen species (ROS) and lipid droplet accumulation was abolished. Furthermore, PA-mediated down-regulation of lipid metabolism genes was reversed by the treatment of HS-Fx. By long non-coding RNAs (lncRNAs) screening, we found that PA-induced increases in the targeted lncRNAs were also decreased upon the treatment of HS-Fx. Silenced these lncRNAs corresponded to the decrease in lipid droplet accumulation of ADSCs induced by PA. ADSC from obese patient would be a direct and meaningful model cell than cell lines from other species to investigate the development of obesity-related diseases and their treatments. HS-Fx showed anti-obesity capability through modulating the elevation of ROS and down-regulation of lipid metabolism genes induced by PA, and the upstream signaling might be critically resulted from the expression of lncRNAs.

Introduction

Brown seaweeds have been the mainstay of the island nation diet and are also addressed as traditional Chinese medicine for over hundred decades. Fucoxanthin (Fx), one of the most abundant carotenoids found in brown algae, shows a source of marine components with potential applications in medicine and health benefits. It has been reported that fucoxanthin showed chemoprevention capability against cancer, immunomodulation property, and antioxidation.[1-3] Furthermore, since 2004 even earlier, Dr. Miyashita and colleagues in Japan, one of the outstanding research teams that are investigate the function of brown seaweeds, has been suggesting that fucoxanthin shows anti-obesity beneficial.[4, 5]

Being overweight and obese is associated with numerous comorbidities and is a critical risk factor for several of the leading causes of death, including cardiovascular disease, diabetes mellitus, hypertension, and many types of cancer.[6-8]

[a]*Department of Pediatrics Shuang Ho Hospital, Taipei Medical University, 291, Jhongjheng Rd., Jhonghe Dist., New Taipei City 23561, Taiwan. E-mail: pedkuoyt@s.tmu.edu.tw; Fax: +886-2-22490088 ext. 2507; Tel: TEL: +886-2-22490088*

[b]*Comprehensive Weight Management Centre, Taipei Medical University Hospital, Taipei Medical University, Taiwan*

[c]*School of Nutrition and Health Sciences, College of Public Health and Nutrition, Taipei Medical University, Taiwan*

[d]*Department of Plastic Surgery, Wan-Fang Hospital, Taipei Medical University, Taiwan*

[e]*Seafood Technology Division, Fisheries Research Institute, Council of Agriculture, Keelung, Taiwan*

[f]*Department of Pediatrics, School of Medicine, College of Medicine, Taipei Medical University, Taiwan*

It has been documented that epigenetic modulation of gene transcription by non-coding RNAs closely involves in the development and biological process of organisms.[9,10] Long non-coding RNA (lncRNA) whose transcripts is longer than 200 nt in length is a class of non-coding RNA and itself does not encode any proteins. As compared to microRNAs, the role of lncRNAs in control of metabolism and energy homeostasis remains unclear. Various diseases but relatively less data about the obesity have been involved in aberrant expression of lncRNAs and the related dysregulation of mRNAs.[11]

Adipose-derived stem cells (ADSCs) have been paid more and more attention that show potential differentiation capability such as adipogenesis, chondrogenesis and osteogenesis, etc. and possess multipotent functions.[12,13] In addition, fat is the endocrine tissue that secretes a wide range of hormones, cytokines and immune regulatory factors. The accumulation of excess lipids that resulted in chronic inflammation in adipose tissue has been linked to insulin resistance and obesity-related diseases.[14,15] Therefore, it would be the target for investigating the effects of external environment (such as high-sugar, high-fat diet, etc) on the growth and differentiation of adipocytes. More importantly, morbid obese patient-derived ADSC show more significance and specificity for further examination and medication.

Recently, we found that high glucose and fat induced lipid accumulation in hepatocyte, and treatment of antioxidant component ameliorated intracellular lipid droplet accumulation.[16.] In the present study, we investigated the effects of high stability fucoxanthin (HS-Fx) on lipid accumulation in fatty acid treated ADSCs from morbid obese patients. As elevated oxidative stress is closely associated with obesity and exogenous oxidative stress promotes adipogenesis of mesenchymal stem cells,[17] we hypothesized that palmitic acid-induced increase in ROS and lipid accumulation was alleviated by antioxidant HS-Fx. Furthermore, the down-regulation of palmitic acid-mediated lipid metabolism genes was recovered by the treatment of HS-Fx. The upstream signaling transduction might through the modulation of long non-coding RNAs.

Materials and methods

Materials

The high stability fucoxanthin (HS-Fx) was the gift from Dr. P.A. Huang (Seafood Technology Division) and Hi-Q Marine Biotech Company (Taiwan). The extraction method was followed the method mentioned before with technological modification.[18] HS-Fx is a mixture of brown seaweed extract containing about 10% of fucoxanthin that coated directly with polysaccharides of its own. It was dissolved in double-distilled H_2O (ddH_2O) and completed dissolved with stirring at room temperature for 30 min. The supernatant were filtrated through 0.22 μm filter. The filtrate were aliquoted and stored at -20 °C. Palmitic acid (PA, C16:0), Alcian Blue 8GX, Oil Red O, and bovine serum albumin (BSA) were purchased from Sigma Aldrich (Shanghai, China). Other high-grade reagents used in this study were all purchased from commercial companies.

Human adipose tissue

Patients aged from 20-40 with BMI (kg/m^2) > 30 outpatient clinic for weight-loss surgery were enrolled in this study at Comprehensive Weight Management Center, Taipei Medical University (TMU) Hospital. All patients signed informed consent in order to take part in the project. This study was approved by the TMU-Joint Institutional Review Board (TMU-JIRB, No.201306007). Herein, we randomly collected abdominal omental or subcutaneous adipose tissues from three (One male, two female) individuals who accepted weight-loss surgery for the following studies.

ADSC isolation and culture

The procedure of human stromal vascular fraction (hSVF) isolation has been described previously.[12] Briefly, the adipose tissue was rinsed with PBS containing 1% penicillin and streptomycin (PS) to clean up the blood, minced into small pieces, and then incubated in a solution containing 0.075% collagenase type IA (Sigma-Aldrich, St. Louis, MO) and 1% PS for 1 h at 37°C with vigorous shake (100 rpm) in the water bath. After allowing to stand at room temperature for 5 min, the top lipid layer was removed and the remaining liquid portion was centrifuged at 220 g for 10 min at room temperature. After washing twice with PBS, the pellet was treated with RBC Lysis Buffer (eBioscience, San Diego, CA, USA) for 10 min to lyse red blood cells. The remaining cells were suspended in DMEM supplemented with 10% KnockOut™ Serum Replacement (KSR, Invitrogen, San Diego, CA, USA), filtered through a 40-μm cell strainer (BD Biosciences, Bedford, MA), and plated at a density ~5×10^5 cells in a 10-cm dish. After reaching 80% confluence, the cells were harvested with TrypLE™ Select (Invitrogen) and stored in liquid nitrogen at a density of 5×10^5 cells per ml of freezing media (DMEM basal medium, 20% KSR, and 10% DMSO).

Preparation of bovine serum albumin (BSA)-conjugated palmitic acid (PA)

PA-BSA was prepared following the method described previously with slight modification.[19] Stock solution of PA (≥99% purity, Sigma Aldrich) were made using 100% of ethanol as a vehicle and stored at -20°C. The stock solution was freshly incubated with bovine serum albumin (BSA, ≤0.1 ng/mg endotoxin, ≤0.02% fatty acids, Sigma Aldrich) at 50°C overnight, and then added to low-serum (2% FBS) DMEM medium to a final concentration of 200 μM fatty acid:100 μM BSA.

Flow cytometry

Single cell suspensions (2×10^5 cells) were analyzed using a 4-laser FACScalibur (BD Science) and stained with the following antibodies: anti-human CD29, anti-human CD31, anti-human CD34, anti-human CD44, anti-human CD90, anti-human CD105 and anti-human IgG isotype, respectively (eBioscience, San Diego, CA, USA). After washing with PBS, the ADSCs were incubated with secondary antibody which was FITC-conjugated for flow cytometry evaluation. The raw data were further analyzed with DiVa software (BD).

Adipogenesis and chondrogenesis assay

ADSC at passage 5-10 was analyzed to confirm the capacity to differentiate toward the adipogenic and chondrogenic lineages. To induce the respective differentiations, ADSCs were cultured with commercial lineage-specific induction media (Invitrogen, San Diego, CA, USA). To induce adipogenic and chondrogenic differentiation, cultures in respective lineage-specific media (Invirogen STEMPRO® Adipogenesis and Chondrogenesis Differentiation Kit) were maintained at 37 ˚C in 5% CO_2 for 14 days for adipogenesis and 21 days for chondrogenesis, respectively. During the culturing process, the medium was changed every 3 days.

Histological analysis

Oil Red O staining: After treatment, the 6-well plates containing differentiated ADSCs were washed with PBS three times (1 ml/well), fixed with 4% formaldehyde (1 ml/well), sealed to prevent dehydration, and then stored at 4 °C. Subsequently, the fixative was aspirated and the individual wells were stained with 1 ml of freshly prepared 0.3% Oil Red O staining solution for 1 h and then washed five times with water. Upon completion of the photographs and scans, 100% isopropanol was added into each well and the plates were shaken for 2 h at room temperature. Eluates from each well were transferred to individual wells on a 96-well plate for optical density readings at 520 nm. Eluate from a well containing PA-BSA-treated differentiated ADSCs served as a control that was compared to all experimental groups. The subtracted values were then used to represent the inhibitory capability of adipogenesis.

Alcian Blue staining: To stain the chondrogenic differentiated cells, 1×10^6 ADSCs were transferred to a 3-D Petrisoft 35 mm dish (Matrigen, Brea, CA, USA). The cells inside chamber were cultured for 21 days and changed fresh differentiation medium to ensure the complete differentiation. For Alician Blue staining, the cells were fixed for 5 minutes in cooled 4% formaldehyde in PBS. Subsequently, the cells were stained with 1% Alcian Blue (in 3% acetic acid, pH2.5) for 30 min and then washed three times with distilled water.

High stability fucoxanthin (HS-Fx) treatment

Briefly, ADSCs (5×10^5 cells) were loaded in a single well of 6-well plate before the day for treatment. The medium were changed with adipogenic medium and the cells were incubated for 9 days (refresh medium every 3 days), and on the 10^{th} and 12^{th} day, fresh medium containing PA-BSA (200 μM) and various concentrations of HS-Fx were added. Controls received an equal volume of ddH_2O vehicle containing 100 μM BSA. The cells were harvested on the 14^{th} day for further study.

Dye exclusion cell viability assay

Cell viability was examined by trypan blue exclusion assay. After treatment, the cells were harvested and incubated in 0.2% trypan blue (Sigma Aldrich) for 3 min and then counted under phase-contrast microscopy. Blue cells were considered as dead cells. The cell viability was expressed as a percentage of the viable cells to the total counted cells. Control that treated with vehicle was expressed as 100%.

Oxidative stress evaluation

After 10 days differentiation duration, the differentiated ADSCs were treated with various concentrations of HS-Fx for 4 days. At the end of incubation, the cells were harvested and then incubated with 2',7'-dichlorofluorescin ($DCFH_2$-DA, 20 μM, final concentration) for 30 min after washing with cold PBS. Finally, the cells were resuspended in 500 μl PBS and then analyzed by flow cytometry. Fluorescence was measured at an excitation wavelength of 488 nm and an emission wavelength of 530. Data were collected from 10,000 cells and evaluated by CELL QuestTM and ModFit softwares.

Reverse transcription PCR analysis

The differentiated ADSCs were treated with HS-Fx for 4 days, the total cellular RNA were isolated using MasterPure™ RNA Purification Kit (Epicentre Biotechnologies, Madison, WI, USA) according to the manufacture's protocols, and then quantified by an absorbance at 260 nm. RNA purity was determined using A260/A280 ratio (average $\geqq$ 1.8). Total RNA from each specimen was first reverse-transcribed into cDNA using a MMLV Reverse Transcriptase 1st-Strand cDNA Synthesis Kit (Epicentre Biotechnologies). PCR amplification was performed by Step One Plus (ABI prism 7500). Real-time PCR was performed using a Fast Start SYBR Green master mix kit (Applied Biosystems). The PCR reaction included the following components: each primer at a concentration of 10 μM, diluted cDNA template, and Fast Start SYBR Green master mix and running 40 cycles (all the primer sequences used were shown in Table 1). Each cDNA sample was run in triplicate, and GAPDH primers as an internal control were included in each run to correct sample-to-sample variation and to normalize mRNA levels. The relative mRNA level was calculated according to the comparative $2^{-\Delta\Delta Ct}$ method.

Long non-coding RNA array

The sample preparation were performed based on the protocol of the Human lncRNA Profiler^{TM} qPCR Array Kit (SBI, Mountain View, CA, USA). Briefly, 2 μg RNA was mixed with PolyA buffer, $MnCl_2$, ATP, and PolyA Polymerase and incubated for 30 min at 37°C. Then mixed with Oligo dT Adapter and heated for 5 min at 60°C. After that RT Buffer , dNTP, DTT, Random Primer Mix, and Reverse Transcriptase were added into the mixture and incubated for 1 h at 42°C. Finally, the lncRNA cDNA was heated for 10 min at 95°C and stored at -80°C for further study. Mastermix (contained SYBR Green qPCR Mastermix buffer, lncRNA cDNA and RNase-free water) and lncRNA Primer were mixed and the qPCR standard protocol was followed: one cycle of 50°C for 2 min, followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min. Data were read at the step of 60 °C for 1 min and the relative lncRNA level was calculated according to the comparative $2^{-\Delta\Delta Ct}$ method as compared to endogenous reference RNAs.

Silencing study of lncRNA on adipogenesis of ADSCs

We examined the effect of loss-of-function (LOF) of targeted lncRNA on the differentiation of ADSCs upon the treatment of PA-BSA. The cells were transfected with anti-sense oligonucleotides (ONT) against targeted lncRNAs or non-targeting control (Dharmacon), respectively, 2 days before differentiation, and followed by the treatment of PA-BSA according to the methods mentioned above. ONT (20 nM) was transfected into the cells with DharmaFECT^{TM} Transfection Reagents. The overall effects of silencing lncRNA on ADSCs adipogenesis was verified by ORO stain.

Statistical analysis

All data are expressed as the mean ± standard error. Significant differences between two groups are determined by Student *t*-test, one-way analysis of variance (ANOVA) or two-way ANOVA (SPSS 11.0). A P value of <0.05 is considered statistically significant.

Results

Isolation and characterization of ADSCs

The isolated ADSCs were cultured in restriction serum condition to maintain the stemness and differentiation capability. Fig. 1A shows the morphology of harvested ADSCs. Unsorted ADSCs were analyzed for growth, differentiation and morphological appearance; there were no differences noted between the patients in terms of the above characteristics. Adipogenesis and chondrogenesis were assessed by Oil Red O staining and Alizarin Blue staining, respectively. The results showed that the ADSCs have potential and multilineage differentiation capability that at least toward adipogenic and chondrogenic cell lineages Fig. 1B and 1C). A cell surface marker profile was evaluated on the isolated ADSCs over a culturing period from passage 5 to passage 6. The data showed that the expression levels of CD29, CD34, CD44, CD90 and CD105 were initially conserved (Fig. 1D). However, the level of CD31 was relatively lower.

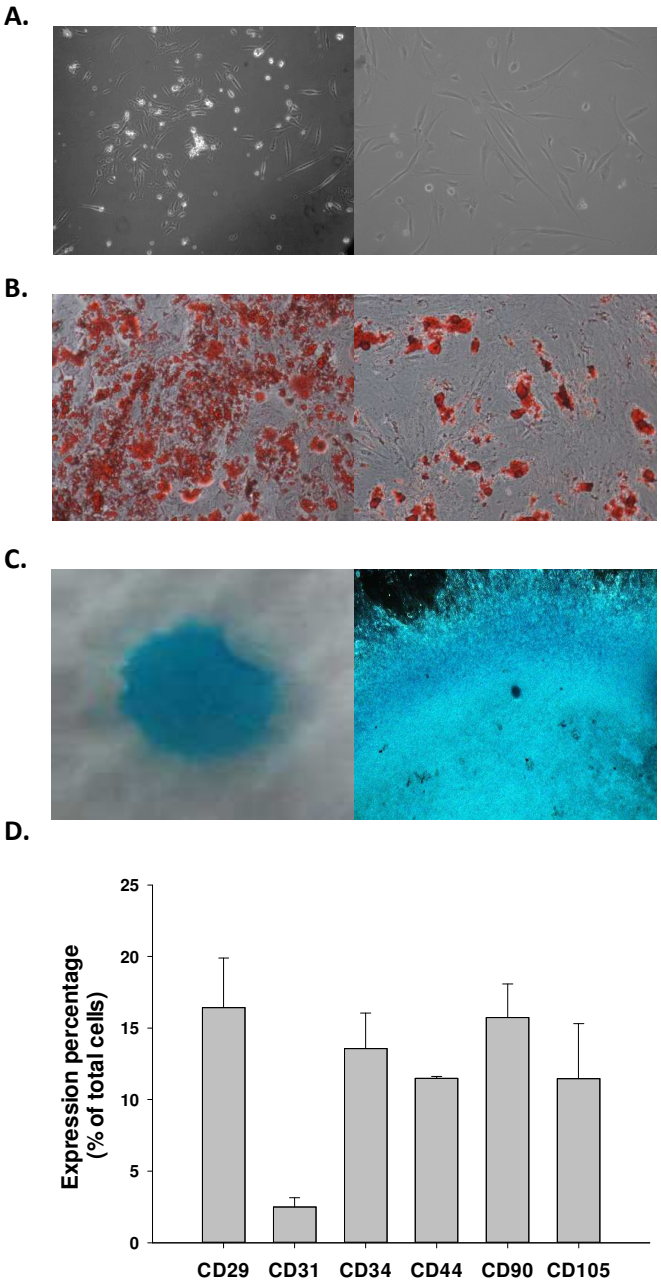

Fig. 1. Human ADSCs with stemness CD markers show adipogenesis and chondrogenesis capabilities. (A) Morphology of ADSCs. ADSCs were incubated with adipogenesis (B) and chondrogenesis (C) induction medium and stained with Oil-Red O and Alcian Blue, respectively. (D) ADSC showed specific stemness differentiation surface

markers. ADSCs were executed adipogenesis and chondrogenesis for 14 days and 21 days, respectively. Magnification is ×100 (left panel) and ×200 (right panel), respectively. Data were shown as means±SEM for three independent studies.

High stability fucoxanthin inhibits palmitic acid-induced oxidative stress but without affects the cell viability

To investigate whether treating cells with palmitic acid results in the production of free radicals during adipogenesis and the effects of HS-Fx, we evaluated the cellular reactive oxygen species (ROS) content by flow cytometry. First, at the end of 4 days incubation, the cell viability was determined by trypan blue dye exclusion assay. The data showed that PA-BSA treatment slightly enhanced the cell viability, and co-treatment of HS-Fx did not noticeably affect the cell viability even at high dose (500 μg/mL, Fig. 2A). The results also indicated that basal ROS levels increased by approximately 95% in ADSCs cells treated with PA-BSA for 4 days, and co-treatment with HS-Fx dose-dependently and significantly ($p<0.05$) inhibited ROS formation (Fig. 2B).

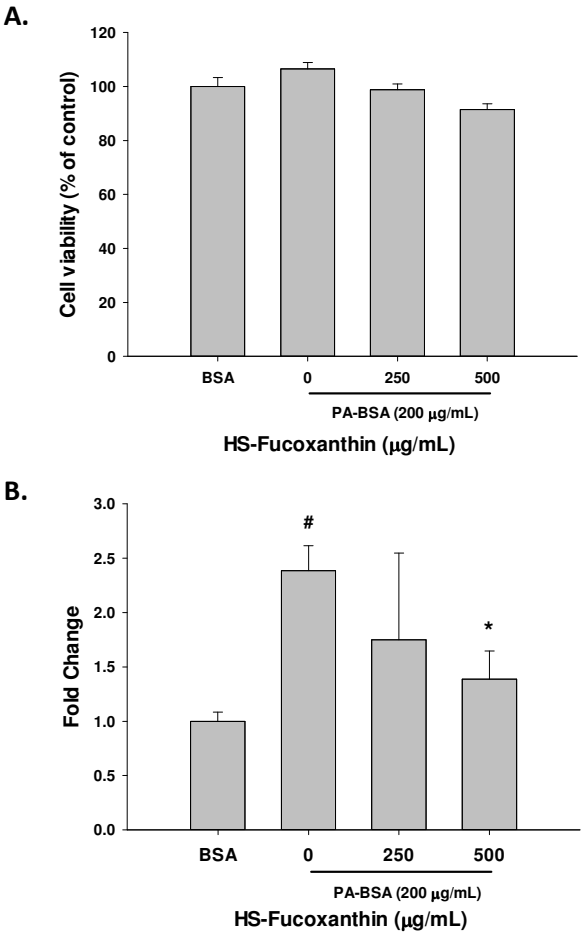

Fig. 2. Non-cytotoxic HS-Fx shows antioxidant capability against palmitic acid-induced increase in ROS in ADSCs. (A) HS-Fx shows no significant cytotoxicity against ADSC upon the treatment of PA-BSA. (B) HS-Fx shows dose-dependent effects on PA-BSA induced elevation of ROS. Data were shown as means±SEM for three independent studies. # and * represent $P<0.05$ versus BSA or PA-BSA treated along group, respectively.

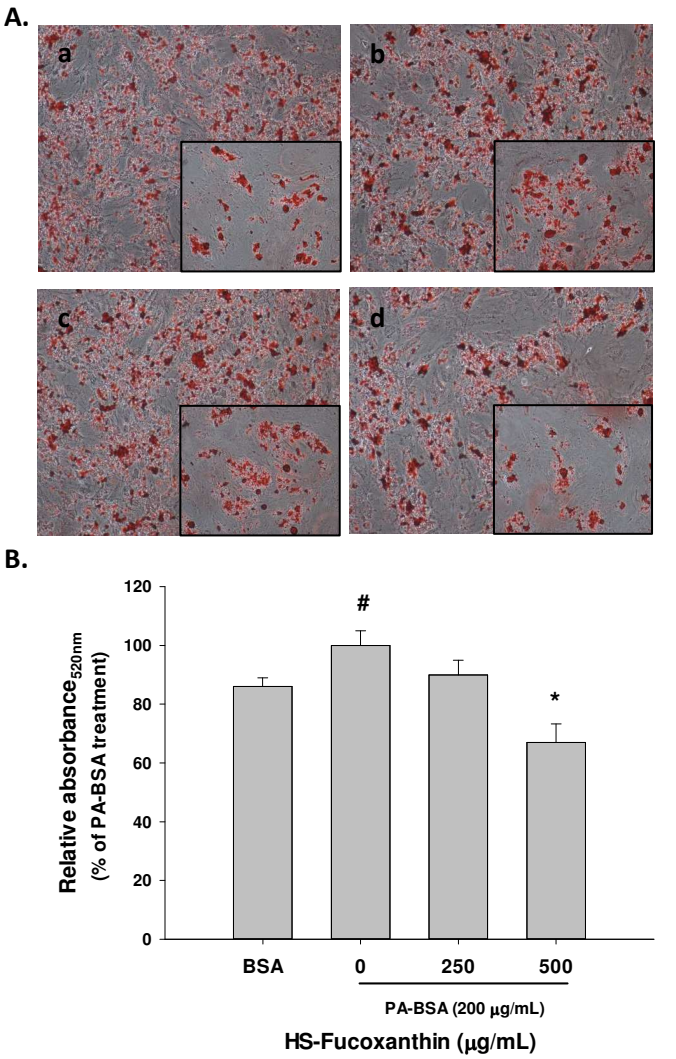

Fig. 3. HS-Fx dose-dependently decreases the accumulation of lipid droplet in ADSC upon the treatment of palmitic acid. (A) ORO stain of ADSCs treated with BSA (a), PA-BSA (b) or HS-Fx and PA-BSA co-treatment (c and d). (B) Data were shown as means. SEM for three independent studies. # and * represent $P<0.05$ versus BSA or PA-BSA treated along group, respectively.

High stability fucoxanthin inhibits palmitic acid-induced lipid accumulation

After 10 days of differentiation from preadipocytes into adipocytes (day 1st to 10th), ADSCs were treated with HS-Fx (0-500 μg/mL) for another 4 days (day 10th to 14th) during the lipid accumulation upon the treatment of PA-BSA. Oil Red O staining was performed to examine the content of lipid in

mature ADSCs adipocytes. As Fig. 3 shows, PA-BSA treatment significantly ($p<0.05$) increased in lipid accumulation. However, significantly ($p<0.05$) lower lipid accumulation was observed at high concentration of HS-Fx (500 μg/mL) compared to the PA-BSA treated group (Fig. 3B). Low concentration of HS-Fx (250 μg/mL) only slightly decreased lipid droplet formation.

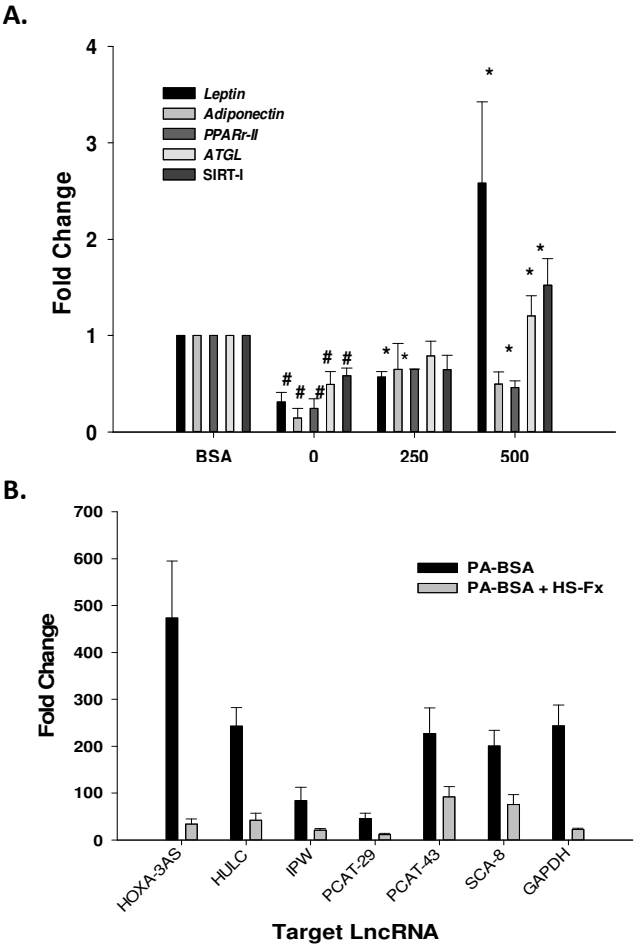

Fig. 4. HS-Fx reverses the decrease in lipid metabolism genes through modulation of lncRNAs. (A) ADSCs were treated with various concentrations of HS-Fx upon the treatment of PA-BSA. qPCR assay was performed for determination of mRNA expression of targeted gene. (B) Human lncRNA Profiler™ qPCR Array Kit was executed for detection of lncRNAs under the treatment of PA-BSA in the present of HS-Fx. Data were shown as means±SEM for three independent studies. # and * represent $P<0.05$ versus BSA or PA-BSA treated along group, respectively.

High stability fucoxanthin modulates mRNA expressions of lipid metabolism-related genes in ADSCs

To further understand the mechanisms underlying the inhibition of lipid accumulation by HS-Fx, mRNA from treated ADSCs were examined by real-time PCR. A significant decrease in the *PPARr-II* gene expression was observed in PA-BSA treated group (Fig. 4A). Furthermore, the genes involved in adipocyte differentiation and lipid metabolism (*Leptin*, *Adiponectin*, *ATGL* and *SIRT1*) were significantly decreased in mRNA levels upon the treatment of PA-BSA, but were further reversed by HS-Fx co-treatment.

High stability fucoxanthin inhibits PA-BSA induced lipid accumulation through modulation of lncRNA

We further investigated long non-coding RNA (lncRNA) expression in adipocytes during adipogenesis by Human LncRNA Profiler to identify specific lncRNA that modulate adipocyte function upon HS-Fx treatment. Changed expression levels of lncRNAs were confirmed by quantitative real-time polymerase chain reaction (qPCR). Among the lncRNAs whose expression levels altered after 4 days PA-BSA treatment of ADSCs, six much up-regulated lncRNAs (HOXA-3AS, HULC, IPW, PCAT-29, PCAT-43, and SCA-8) were observed. Interestingly, co-treatment of the cells with HS-Fx dramatically decreased the levels of these lncRNAs (Fig. 4B). To confirm the role of lncRNA in PA-BSA induced adipocyte hypertrophy, we further randomly selected four anti-sense inhibitors against lncRNAs (HOXA-3AS, HULC, and IPW, SCA-8) to transfect and examined the lipid accumulation of ADSCs. Inhibition of these lncRNAs by their anti-sense inhibitors showed decreased in lipid droplet accumulation of ADSCs upon the treatment of PA-BSA (Fig. 5A and 5B).

Discussion

Fucoxanthin, a carotenoid pigment is found abundantly in brown seaweed. It has been reported to show antioxidant, anti-obesity and anti-diabetic activities. As a type of carotenoids, fucoxanthin is sensitive to degradation by external agents, such as heat, low pH and light exposure. High stability fucoxanthin (HS-Fx) is a gift from Seafood Technology Division (Taiwan), and it is a more stable fucoxanthin which with edible polysaccharides. More than 86% of HS-fucoxanthin (1% w/v) remained unchanged in the solution after storage at room temperature for 8 h, and fucoxanthin standard only remained 2% under the same condition (Fig. S(A-C)). Furthermore, when stored at 4 °C for two days and -20°C for 28 days, less than 50% and 80% of fucoxanthin was detected, respectively, but there was still more than 90% HS-Fx detected under the related condition. Actually, polysaccharides, such as chitosan scaffold, have been used for improving the stability and cellular uptake of oral biocomponents delivery.[20] Much recently, polysaccharide fucoidan derived from brown seaweed, might show functional properties for drug delivery and therapeutic treatment.[21] Unlike Xanthigen which also claimed for the weight loss benefit, HS-Fx only contains the components derived from brown seaweed extract containing 10% of high stability fucoxanthin and other edible polysaccharides. Thus, we

supposed that HS-Fx might improve the bioavailability and stability; however, the extraction yield is still a critical factor to overcome.

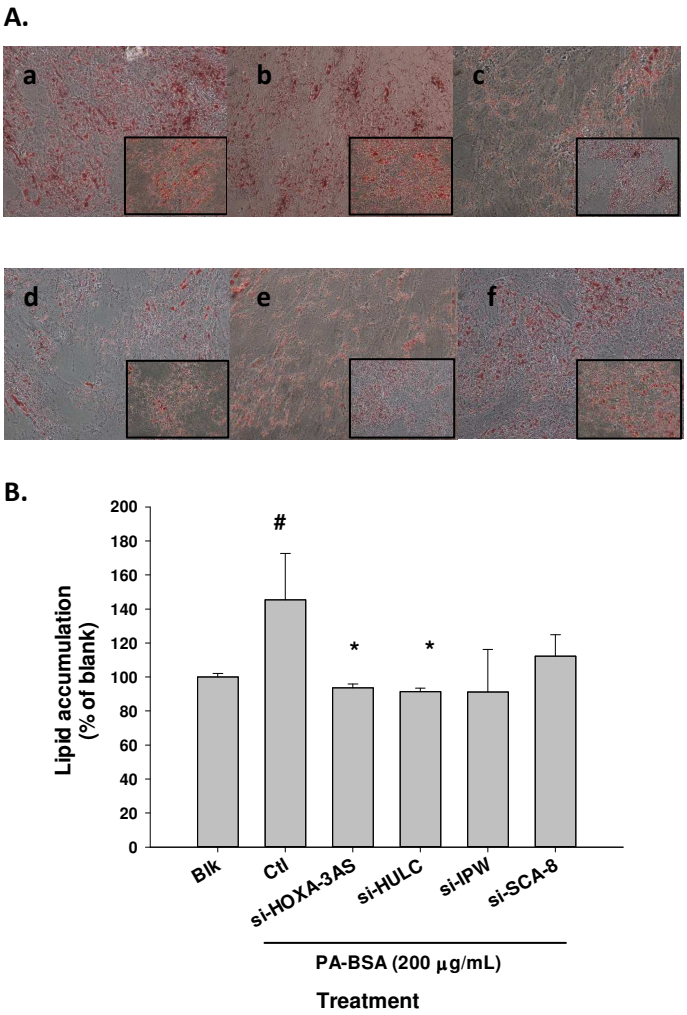

Fig. 5. Silences specific long non-coding RNAs abolish the lipid droplet accumulation in palmitic acid treated ADSCs. (A) Magnification is ×100 and ×200 for adipocytes treated with BSA (a), PA-BSA (b), si-HOXA-3AS (c), si-HULC (d), si-IPW (e), and si-SCA-8 (f). (B) Data were shown as means±SEM for three independent studies. # and * represent $P<0.05$ versus BSA or PA-BSA treated along group, respectively.

Both fucoxanthin and its deacetylated product, fucoxanthinol, show biological efficacy in metabolic modulation. Since 2006, the inhibitory effects of fucoxanthin and fucoxanthinol against adipogenesis have been demonstrated.[22, 23] In a noted human study, after 1 week of uptake of fucoxanthin, the results showed that except fucoxanthinol, no fucoxanthin and amarouciaxanthin A was detected in the plasma, the possible argument was that some components (e.g., dietary fibre) in algal matrix inhibited the intestinal absorption of fucoxanthin.[24] The bioavailability of fucoxanthinol is higher than that of fucoxanthin in the body. However, the daily oral administration of fucoxanthin for 1 week showed that a small amount of fucoxanthin was not metabolized and was detectable in the liver, lung, kidney, heart, spleen, and adipose tissue of the mice.[25] In addition, fucoxanthin and fucoxanthinol inhibited both lymphatic triglyceride absorption and the increase of triglyceride concentration in systemic blood, likely due to their inhibitory effects on lipase activity in the gastrointestinal lumen.[26] Thus, both fucoxanthin and its metabolites show biological benefits.

Although there are several *in vitro* studies discuss the inhibitory effects of fucoxanthin on lipid accumulation, they are almost based upon the model of 3T3-L1, a *Mus musculus* cell line or rodent animal that are widely used in this field.[4, 27, 28] In the present study, also to be the first study, to evaluate the effects of fucoxanthin on saturated fatty acid-induced lipid accumulation in human adipose-derived stem cells (ADSCs), which were specific from morbidly obese patients, and from which we could more understand the potent and cause of adipogenesis of these patients. Recently, our team leader, Dr. Wang, has revealed some correlation between genetic risk and obesity from patients received bariatric surgery, such as laparoscopic adjustable gastric banding (LAGB) and laparoscopic mini-gastric bypass (LMGB).[29,30] We further harvested the fresh adipose tissue and cultured ADSCs from randomly-selected adult-onset obese patient undergoing bariatric surgery for performing this study.

The American Medical Association has officially recognized obesity as a disease. Obesity is a public health crisis affecting approximately more than one third of Americans and costing the healthcare system more than $190 billion annually. It has been suggested that the total healthcare costs attributable to obesity could reach near to trillion by 2030.[15] Studies have shown that dietary fatty acids distinctly influent the transcriptional profiles in human peripheral blood mononuclear cells and subcutaneous adipose tissue, but the similar study on rodent cell line did not showed complete directional concordance of genes expression compared to the human studies. The reason might due to the difference between the species and cell types.

Recently, it has just been reported that palmitoleic acid, but not palmitic acid, increases white adipocyte lipolysis and lipase content in a PPARα-dependent manner, and decreases lipid accumulation.[31] Our recent finding also showed that high-palmitic acid and high-glucose induced lipid accumulation in hepatocytes. Studies also showed that up-regulation of the *Sirt1* and *PCG-1α* genes in white adipose tissue might against high-fat diet induced obesity and insulin resistance.[32] Sirtuin 1 (SIRT1) is a nicotinamide adenine dinucleotide (NAD+)-dependent protein deacetylase, which is capable of modifying acetylated proteins, such as peroxisome proliferator-activated receptor γ (PPARγ) coactivator 1α (PGC-1α), and thus allows the protein to transcriptionally activate downstream target genes and regulate metabolism.[33] It has been shown that inhibition of SIRT1 results in the promotion of adipogenesis.[34] Furthermore, adipose triglyceride lipase (ATGL), a key enzyme involves in the lipolysis, would be enhanced by phytochemicals and resulted

in the antiobesity benefits.[33] In our study we also found that PA-BSA induced down-regulation of ATGL, SIRT1 and PGC-1α significantly reversed by the treatment of HS-Fx. claimed that several non-coding named transcripts related to hMSC differentiation (TMDs), which displayed distinct transcriptional kinetics during hMSC adipogenesis and/or osteogenesis.[36] By using Microarray or RNA Profiling technology, some studies showed that various lncRNA transcript functioning in adipogenesis.[11, 37] However, there is extremely less research about how food affects lncRNA expression during lipid accumulation.

Long non-coding RNA (lncRNA) whose transcripts is longer than 200 nt in length is a class of non-coding RNA and itself does not encode any proteins. There is relatively less discussion about the function of lncRNA in adipogenesis than other biological process since 2009.[36] Herein, we first investigated the effects of palmitic acid (PA), a kind of saturated long chain fatty acid, on the expression of lncRNA during adipogenesis in ADSC from morbid obesity patients. To survey the functional effects of lncRNAs during lipid accumulation, we performed RNAi mediated loss-of-function (LOF) experiments for four candidate lncRNAs. As compared to control group (BSA-treated cells), we found six highly up-regulated lncRNA upon PA-BSA treatment, and all of them were inhibited by the treatment of HS-Fx. Furthermore, silenced these lncRNAs showed decrease in lipid accumulation upon the treatment of PA-BSA, these results suggested that the inhibitory effects of HS-Fucoxanthin on palmitic acid induced lipid accumulation might through down-regulating lncRNAs that are involved in adipogenesis (Fig. 6.).

Conclusions

In conclusion, this study suggested that (1) human adipose-derived stem cell is a reasonable in vitro model than others for studying how food or disease affect mankind and (2) naturally occurring fucoxanthin showed potent effects of inhibiting lipid accumulation and reversing lipid metabolism gene expression of ADSC upon the treatment of palmitic acid. Much importantly, we found that genetically modulation by targeted lncRNA regulated adipogenesis. Thus, lncRNA might be a critical target for studying the development of morbid obesity and the treatment to the late-onset obesity-induced diseases. The underlying epigenetic mechanisms and the physiological efficacy of fucoxanthin need to be further verified.

Abbreviations

ADSC	adipose-derived stem cell
SVF	stromal vascular fraction
HS-Fx	high stability fucoxanthin
PA	palmitic acid
ROS	reactive oxygen species
lncRNA	long non-coding RNA
PPARγ	peroxisome proliferator activator receptor γ
ATGL	adipose triglyceride lipase
ORO stain	oil red O stain

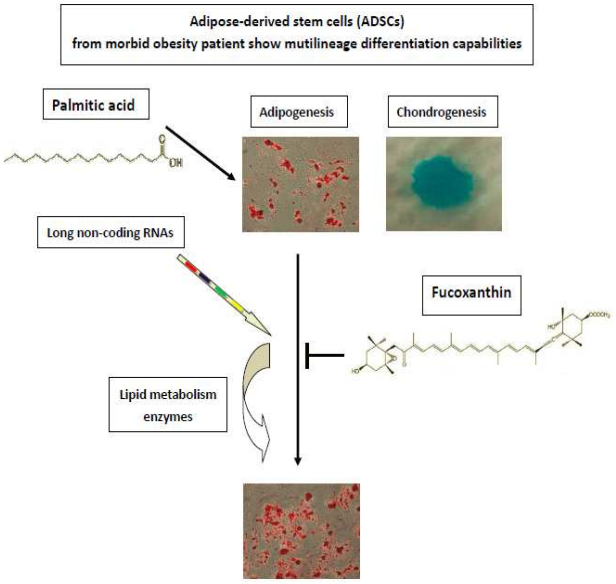

Fig. 6. Supposed mechanism of fucoxanthin mediated down-regulation of lipid accumulation in matured ADSCs from morbid obesity patient induced by palmitic acid through modulation of long non-coding RNA and lipid metabolism enzymes.

Acknowledgements

The research leading to these findings received funding from the Taipei Medical University (TMU101-AE3-Y08). We also thank Po-Li Chen and Pei-Yeh Lee at National Defense Medical Center (Taiwan) for technical support.

References

1 J. Peng, J. P. Yuan, C. F. Wu and J. H. Wang, *Mar. Drugs,* 2011, **9**, 1806-1828.
2 T. Rengarajan, P. Rajendran, N. Nandakumar, M. P. Balasubramanian and I. Nishigaki. *Nutrients*, 2013, **5**, 4978-4989.
3 Y. Zhang, H. Fang, Q. Xie, J. Sun, R. Liu, Z. Hong, R. Yi and H. Wu, *Molecules*, 2014, **19**, 2100-2113.
4 H. Maeda, M. Hosokawa, T. Sashima, K. Funayama and K. Miyashita, *Biochem. Biophys. Res. Commun*., 2005, **332**, 392-397.
5 H. Maeda, M. Hosokawa, T. Sashima, K. Murakami-Funayama and K. Miyashita, *Mol. Med. Rep*., 2009, **2**, 897-902.
6 G. Egger and J. Dixon. *Bio.Med. Res. Int.,* 2014, **2014**, 731685.
7 K. Ghoorah, P. Campbell, A. Kent, A. Maznyczka and V. Kunadian, *Eur. Heart. J. Acute. Cardiovasc. Care*, 2014. Feb 13.
8 E. Grunvald, *Clin. Obstet. Gynecol.,* 2014, **57**, 465-484.
9 R. Bonasio and R. Shiekhattar, *Annu. Rev. Genet.,* 2014, **48**, 433-455.
10 J. W. Kornfeld and JC Bruning. *Front. Genet.,* 2014, **5**, 57.
11 L. Sun, L. A. Goff, C. Trapnell, R. Alexander, K. A. Lo, E. Hacisuleyman, M. Sauvageau, B. Tazon-Vega, D. R. Kelley, D. G. Hendrickson, B. Yuan, M. Kellis, H. F. Lodish and J. L. Rinn, *Proc. Natl. Acad. Sci. U.S.A*., 2013, **110**, 3387-3392.

12 S. Nae, I. Bordeianu, A. T. Stancioiu and N Antohi, *Rom. J. Morphol. Embryol.*, 2013, **54**, 919-924.
13 K. Senarath-Yapa, A. McArdle, A. Renda, M. T. Longaker and N. Quarto, *Int. J. Mol. Sci.,* 2014, **15**, 9314-9330.
14 C. K. Glass and J. M. Olefsky, *Cell. Metab*., 2012, **15**, 635-645.
15 B. Mahgerefteh, M. Vigue, Z. Freestone, S. Silver and Q. Nguyen, *Am. Health Drug Benefits*, 2013, **6**, 423-430.
16 Y. T. Kuo, T. H. Lin, W. L. Chen and H. M. Lee, *Eur. J. Pharmacol.,* 2012, **692**, 10-18.
17 K. V. Tormos, E. Anso, R. B. Hamanaka, J. Eisenbart, J. Joseph, B. Kalyanaraman and N. S. Chandel, *Cell. Metab.*, 2011, **14**, 537-544.
18 T. Sugawara, M. Kushiro, H. Zhang, E. Nara, H. Ono and A. Nagao, *J. Nutr*., 2001, **131,** 2921-2927.
19 J. E. Ulloth, C. A. Casiano and M. de Leon, *J. Neurochem*., 2003, **84**, 655-668.
20 Z. Li, J. Ha, T. Zou and L. Gu. *Food Funct.,* 2014, **5**, 1278-1285.
21A. Purnama, R. Aid-Launais, O. Haddad, M. Maire, D. Mantovani, D. Letourneur, H. Hlawaty and C. Le Visage. *Drug Deliv. Transl. Res.*, 2015, **5**, 187-197.
22 H. Maeda, M. Hosokawa, T. Sashima, N. Takahashi, T. Kawada and K. Miyashita, *Int. J. Mol. Med.,* 2006, **18**, 147-152.
23 T. Aki, M. Yamamoto, T. Takahashi, K. Tomita, R. Toyoura, K. Iwashita, S. Kawamoto, M. Hosokawa, K. Miyashita and K. Ono, *Lipids*, 2014, **49**, 133-141.
24 A. Asai, L. Yonekura and A Nagao, *Br. J. Nutr.*, 2008, **100**, 273–277.
25 T. Hashimoto, Y. Ozaki, M. Taminato, S. K. Das, M. Mizuno, K. Yoshimura, T. Maoka and K. Kanazawa, *Br. J. Nutr.*, 2009, **102**, 242–248.
26 M. Matsumoto, M. Hosokawa, N. Matsukawa, M. Hagio, A. Shinoki, M. Nishimukai, K. Miyashita, T. Yajima and H. Hara, *Eur. J. Nutr*., 2010, **49**, 243–249.
27 S. I. Kang, H. C. Ko, H. S. Shin, H. M. Kim, Y. S. Hong, N. H. Lee and S. J. Kim, *Biochem. Biophys. Res. Commun*., 2011, **409**, 769-774.
28 C. S. Lai, M. L. Tsai, V. Badmaev, M. Jimenez, C. T. Ho and M. H. Pan, *J. Agric. Food Chem*. 2012, **60**, 1094-1101.
29 T. H. Liou, H. H. Chen, W. Wang, S. F. Wu, Y. C. Lee, W. S. Yang and W. J. Lee, *Obes. Surg*., 2011, **21**, 1758-1765.
30 W. Wang, T. H. Liou, W. J. Lee, C. T. Hsu, M. F. Lee and H. H. Chen, *Surg. Obes. Relat. Dis.*, 2014, **10**, 14-22.
31 A. Bolsoni-Lopes, W. T. Festuccia, T. S. Farias, P. Chimin, F. L. Torres-Leal, P. B. Derogis, P. B. de Andrade, S. Miyamoto, F. B. Lima, R. Curi and M. I. Alonso-Vale, *Am. J. Physiol. Endocrinol. Metab*., 2013, **305**, E1093-1102.
32 Y. Zhao, F. Ling, T. M. Griffin, T. He, R. Towner, H. Ruan and X. H. Sun, *J. Biol. Chem.* 2014, **289**, 29112-29122.
33 A. Chalkiadaki and L. Guarente., *Cell. Metab*., 2012, **16**, 180-188.
34 J. Ahn, H. Lee, C. H. Jung, T. I. Jeon and T. Y. Ha, *EMBO Mol. Med*., 2013, **5**, 1602-1612.
35 S. Wang, N. Moustaid-Moussa, L. Chen, H. Mo, A. Shastri, R. Su, P. Bapat, I. Kwan and C. L. Shen, *J. Biol. Chem*., 2014, **25**, 1-18.
36 K. Kikuchi, M. Fukuda, T. Ito, M. Inoue, T. Yokoi, S. Chiku, T. Mitsuyama, K. Asai, T. Hirose and Y. Aizawa, *Nucleic Acids Res*., 2009, **37**, 4987-5000.
37 F. Yi, F. Yang, X. Liu, H. Chen, T. Ji, L. Jiang, X. Wang, Z. Yang, L. H. Zhang, X. Ding, Z. Liang and Q. Du, *RNA Biol*., 2013, **10**, 991-1001.