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Adipo8, a high-affinity DNA aptamer, can differentiate among adipocytes and inhibit intracellular lipid accumulation *in vitro*

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Obesity is primarily caused by the excessive accumulation of white adipose tissues (WAT). We previously obtained an adipocyte-specific aptamer termed Adipo8 *in vitro*. In this present study, this adipocyte-specific aptamer Adipo8 was first chemically modified by introduction of phosphorothioate linkages (PS-linkages) and then conjugated to polyethylene glycol (PEG), we tested whether this modified aptamer could distinguish mature white adipocytes from 3T3-L1 preadipocytes or brown adipocytes. To verify the binding affinity of this aptamer to mature white adipocytes *in vivo* as well as *in vitro*, we tested whether modified Adipo8 could specifically bind to the WAT of Diet-Induced Obesity (DIO) C57BL/6 mice. Finally, we examined the effect of Adipo8 on the adipogenic differentiation of mature white adipocytes. Based on our results, PS-modified aptamer demonstrated its high binding affinity and specificity, and was able to distinguish white adipocytes from 3T3-L1 preadipocytes or brown adipocytes *in vitro*. PS-modified Adipo8 also demonstrated more biostability and prolonged binding time in biological fluids. Additionally, Adipo8 could inhibit adipogenic differentiation of adipose tissue, possibly by inhibiting the expression of PPAR-γ in adipose tissue. This modified aptamer holds great promise as a stable molecular recognition tool for targeted delivery to adipocytes and has potential in the treatment of obesity.

DNA aptamer, adipocyte, modification, adipogenic, PPAR-γ

1 Introduction

Obesity is a common health risk, and its prevalence is increasing globally. Robust evidence indicates that obesity is associated with an increased incidence of cardio cerebral vascular disease, type 2 diabetes mellitus, and certain types of cancer [1]. The available treatment options remain less than optimal, as obesity continues to become more common among various populations [2]. Since the accumulation of excess white adipose tissue (WAT) plays a dominant role in the progression of obesity, WAT represents one of the most important therapeutic target tissues [3]. Some small-molecule drugs, which are specially targeted to adipose tissue, can regulate lipid metabolism *in vitro*, but they lack specificity, and they have toxic side effects. Therefore, further research is needed to identify adipocyte-specific biomarkers so that molecular tools can be developed for the targeted drug delivery specific to adipose tissue.

Systematic Evolution of Ligands by Exponential Enrichment (SELEX) is an established method used to select short strands of nucleotides, termed aptamers, which can recognize ligands with high specificity and binding affinity [4]. Nucleic acid aptamers are able to be selected from pools of random-sequence oligonucleotides to bind an extensive range of biomedically relevant proteins with specificities and affinities that are comparable to antibodies. Compared with protein therapeutics, aptamers exhibit significant advantages over antibodies as they are easier to produce and modify and have low immunogenicity and biotoxicity [5–9].

Cell-SELEX is a method that can generate aptamers that can bind specifically to a cell type of interest [10]. Com-

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pared with protein-based SELEX, cell-SELEX can be proceeded without prior knowledge of the types of proteins or the molecular signatures on the cell surface, providing an effective approach for the discovery of new biomarkers of target cells without damaging the native conformation and biological function of the target molecules [11–13].

The Liu's group [14] reported the use of cell-SELEX to select an aptamer termed Adipo8, which can specifically recognize mature adipocytes with high affinity. However, aptamer instability often hampers their therapeutic application to *in vitro* and *in vivo* conditions [15]. The circulation half-life of aptamers, such as Adipo8 can be reduced by enzymatic-dependent nuclease degradation in biological fluids. To solve this problem, many chemical modification strategies have been adopted to resist the nuclease activities in order to prolong the half-life of aptamers, such as the introduction of fluoro (–F), amino (–NH_2), or *O*-methyl (–OCH_3) at the 2-position of ribose sugar, high molecular mass polyethylene glycol (PEG), or phosphorothioate linkages (PS-linkages) [16–21]. Compared with the unmodified aptamers, studies have shown the prolonged half-life of modified functional aptamers in biological fluids, which can even havebetter specificity and binding affinity to their targets [22–24].

Herein, we attempted to chemically modify Adipo8 by introducing PS-linkages and conjugation with PEG. In comparison with unmodified Adipo8, we tested whether chemically modified Adipo8 could also exhibit high binding affinity to mature white adipocytes and tested whether it had a longer lifetime *in vitro* or *in vivo*. Subsequently, we preliminarily examined the effects of Adipo8 on the adipogenic differentiation of white adipocytes. Based on our findings, this modified aptamer may also serve as an efficient tool for adipocyte biomarker discovery, or even for the treatment of adipocyte-related metabolic diseases relied on the targeted delivery of therapeutic drugs.

2 Experimental

2.1 Materials and reagents

The 3T3-L1 preadipocytes (Mouse 3T3-L1 preadipocytes) were obtained from ATCC (American Type Culture Collection, Manassas, VA, USA). Mature brown adipocytes were a gift from Feng Liu (Dept. of Metabolism, Central South University, Changsha, China). Dulbecco's modified Eagle's medium (DMEM), calf serum (CS), fetal bovine serum (FBS), Oil Red O, phosphate-buffered saline (PBS), Dulbecco's phosphate-buffered saline (DPBS), bovine serum albumin (BSA), and penicillin-streptomycin solution were all purchased from Gibco-Invitrogen, while 3-isobutyl-1-methyxanthine (IBMX), dexamethasone (DEX), insulin, dimethylsulfoxide (DMSO), formaldehyde and *D*-glucose were purchased from Sigma-Aldrich (USA). Polyvinylidene difluoride (PVDF) membranes were purchased from Thermo Scientific (USA). Trypsin, EDTA, Tween and SDS (sodium dodecyl sulfate) were purchased from Sinopharm Chemical Reagent Corp, China Library and the HPLC-purified oligonucleotides, both unmodified and PS-modified, were purchased from Shanghai Biotech (China).

2.2 Cell culture

The 3T3-L1 preadipocytes were maintained in DMEM supplemented with 10% calf serum in a 10% CO_2 incubator at 37 °C. Previously published articles were followed to differentiate cells [14]. In brief, 3T3-L1 cells were cultured until they reached confluence in 10% calf serum/DMEM. 48 h after reaching confluency (designed day 0), cells were induced to differentiate with DMEM supplemented with MDI media (DMEM containing 10% FBS, 1 μmol/L dexamethasone, 0.5 mmol/L 3-isobutyl-1-methylxanthine, and 1.67 μmol/L insulin). Then 2 days later (day 2), the medium was changed to DMEM containing 10% FBS and 1.67 μmol/L insulin. After 48 h (day 4), the medium was replaced with DMEM with 10% FBS. Cells were subsequently "re-fed" every 48 h with DMEM with 10% FBS until the full differentiation was achieved.

2.3 Circular dichroism (CD) spectroscopy

To determine the structure of PS-modified Adipo8, 10 mmol/L Adipo8 was dissolved in PBS buffer for CD analysis, using a CD-MOS-500 (Biologic). The CD spectrum was recorded in the range 190–300 nm at both 4 and 37 °C. CD spectrum analysis was performed on a MOS-500 spectropolarimeter (Biologic) using a 1-cm path length cuvette. PBS buffer was used as a blank, by which spectral data for Adipo8 were corrected.

2.4 Stability of Adipo8 against nucleases in serum containing medium

In this test, 10 μmol/L unmodified and PS-modified Adipo8 were cultured in DMEM media supplemented with 10% FBS at 37 °C. At different time (0, 1, 2, 6, 12 and 24 h), 25 μL of samples were removed and instantly set to −80 °C in order to minimize unnecessary degradation. Then samples were used for 12% denaturing polyacrylamide gel electrophoresis (PAGE). Gel densitometry and gene tools analysis software was introduced to measure and analyze band densities.

2.5 Analysis of cells bound to Adipo8 by confocal imaging

For confocal imaging, the attached adipocytes were detached using 0.02% EDTA at 37 °C for 15 min, followed by resuspension and centrifugation. Thereafter, cells were

washed three times with 1000 μL washing buffer (WB). After that, 3-end Cy3-labeled PS-modified Adipo8 and unmodified Adipo8 were incubated with cells (5×10^5) in binding buffer (BB) for 40 min at 37 °C in the dark. After washing three times with 1000 μL WB, cells were resuspended in 200 μL WB and set to an Olympus FV1000 confocal microscope (Japan) for confocal imaging.

2.6 Animals and *in vivo* experimental protocols

Four-week-old male C57BL/6 mice were purchased from Shanghai SLAC Experiment Animal Company. All procedures performed on animals complied with the guidelines of the Chinese Council of Animal Care. All experimental animals were approved by the Animal Care and Use Committee of Xiangya Hospital, Central South University of China. After acclimation, 72 mice were fed on regular chow for 2 weeks, after which they were randomly divided into two groups (n=12 in the chow control group, n=60 in the high-fat diet group). In the chow control group, mice were fed regular chow (control group), and the other group was fed a high-fat diet (HFD) consisting of 35% fat, 20% protein and 36.5% carbohydrate (Bio-Serv, Frenchtown, NJ, USA). Body weight, food intake, water intake, and blood glucose levels were measured from non-fasting animals 2 or 3 times per week throughout the study. After 8 weeks on these diets, the Diet-Induced Obesity (DIO) mouse model was successfully established.

To investigate the specific binding of Adipo8 to adipose tissue *in vivo*, we randomly selected 4 DIO mice from the HFD group. The DIO mice received intraperitoneal injections of modified Adipo8 at a concentration of 125 nmol/kg. Then, 1 h after injection, DIO mice were sacrificed and fresh segments of liver, kidney, skeletal muscle and epididymal adipose were frozen. They were then embedded in optimal cutting temperature (OCT) compound. Obtained sections were fixed with acetone for 10 min at normal temperature and washed for H&E or fluorescence aptamer staining.

Additionally, we randomly selected 24 DIO mice from the HFD group and randomly divided them into two groups (n=12 each). Each HFD group respectively received intraperitoneal injections of modified or unmodified Adipo8, also at a concentration of 125 nmol/kg. From this cohort 2 DIO mice were randomly selected and removed at each time point (30 min, 1, 2, 4, 8 and 24 h), and fresh segments of epididymal adipose were frozen for either H&E or fluorescence aptamer staining.

2.7 Oil-Red-O staining

Preadipocytes were seeded at a density of 2×10^4 cells per well, and differentiation was carried out as previously described. Adipocytes were incubated with modified Adipo8 on days 4, 6 and 8 after starting differentiation at a concentration of 250 nmol/L. Then 48 h after each time point, Oil-Red-O staining was carried out, after which the cells were washed gently two times, then were fixed with 4% fresh formaldehyde/PBS for 1 h. The adipocytes were then stained with 0.6% (w/v) filtered Oil Red O solution for 2 h at room temperature. The retained Oil Red O dye in cells was eluted with 600 μL/well isopropanol, and all procedures were performed based on the manufacturer's instructions. The pictures were taken with an inverted phase contrast Olympus microscope and were analyzed by Image J software. Micrographs were obtained from triplicate samples.

2.8 Western blot analysis

Total proteins from adipocytes were extracted in immunoprecipitation assay buffer with protease inhibitors. Samples were centrifuged at 12000 r/min for 10 min, and the supernatants were collected for western blotting. For western blotting, 20 μg protein lysate was separated by SDS-PAGE and then transblotted onto a PVDF. Primary antibodies against PPAR-γ were purchased from Cell Signaling (Beverly, USA). GAPDH antibodies (Cell Signaling) were used to control for protein sample loading. A secondary antibody conjugated to horseradish peroxidase was applied in the chemiluminescence procedure (Immobilon Western, Millipore, USA). All the western blotting procedures were performed based on the manufacturer's instructions with three repetitions.

2.9 Statistical analysis

All data were expressed as means±SD. Independent t-tests were used for statistical analysis through SPSS 19.0 software package. A difference was considered to be statistically significant if it reached a P<0.05.

3 Results and discussion

3.1 Characterization of modified Adipo8

Mammals have two main types of adipose tissue, white adipo tissue (WAT) and brown adipo tissue (BAT). These two tissues have divergent roles in whole-body metabolism. BAT is important for cold- and diet-induced thermogenesis, whereas WAT is well known for its role in energy storage and is also a key target tissue for drug delivery in preventing and treating obesity [25]. One of the biggest risk factors in obesity treatment is the inability to specifically distinguish WAT from various tissues, especially BAT [26]. We have reported the use of cell-SELEX to select an adipocyte-specific aptamer termd Adipo8. The specificity and affinity of Adipo8 for mature white adipocytes that were differentiated from 3T3-L1 preadipocytes has previously been con-

firmed [14]. Herein, we first modified Adipo8 with PS-linkages and conjugated the sequence with PEG to test the possibility that modified Adipo8 could distinguish mature white adipocytes from 3T3-L1 preadipocytes or brown adipocytes. Detailed sequences are shown in Table 1. A randomized library, unmodified Adipo8 and modified Adipo8 were each separately incubated with 3T3-L1 preadipocytes, differentiated mature adipocytes, as well as differentiated brown adipocytes. In Figure 1(A), no bound fluorescence signal could be observed in either 3T3-L1 preadipocytes or differentiated brown adipocytes in the randomized library group. By contrast, both the unmodified and PS-modified Adipo8 groups exhibited high specific binding to mature white adipocytes. Thus, our study provides a new way of distinguishing mature white adipocytes from both preadipocytes and mature brown adipocytes *in vitro*, allowing this aptamer to be deployed for use in the targeted delivery of drugs to mature white adipocytes.

Although aptamer selection processes are generally performed at 4 °C, Adipo8 should be able to deliver drugs *in vivo* at 37 °C. Therefore, we tested whether temperature could affect the binding ability of unmodified and PS-

Table 1 Detailed sequences of unmodified Adipo8 and PS-modified Adipo8 conjugated to PEG

	Sequences
Unmodified Adipo8	ATGAGAAGCGTCGGTGTGGTTAAACACGGAACGAAGGTGCAGGAAGATTTGTCGATG CGGTGCCTGAGCGGGCTGGCAAGGCGCATA
Modified Adipo8	PEG-A*TGAGAAGCGTCGGTGTGGTTAAACACGGAACGAAGGTGCAGGAAGATTTGTC [a] GATGCGGTGCCTGAGCGGGCTGGCAAGGCGCAT*A

a) The "*" indicates the position of a phosphorothioate (PS) modification.

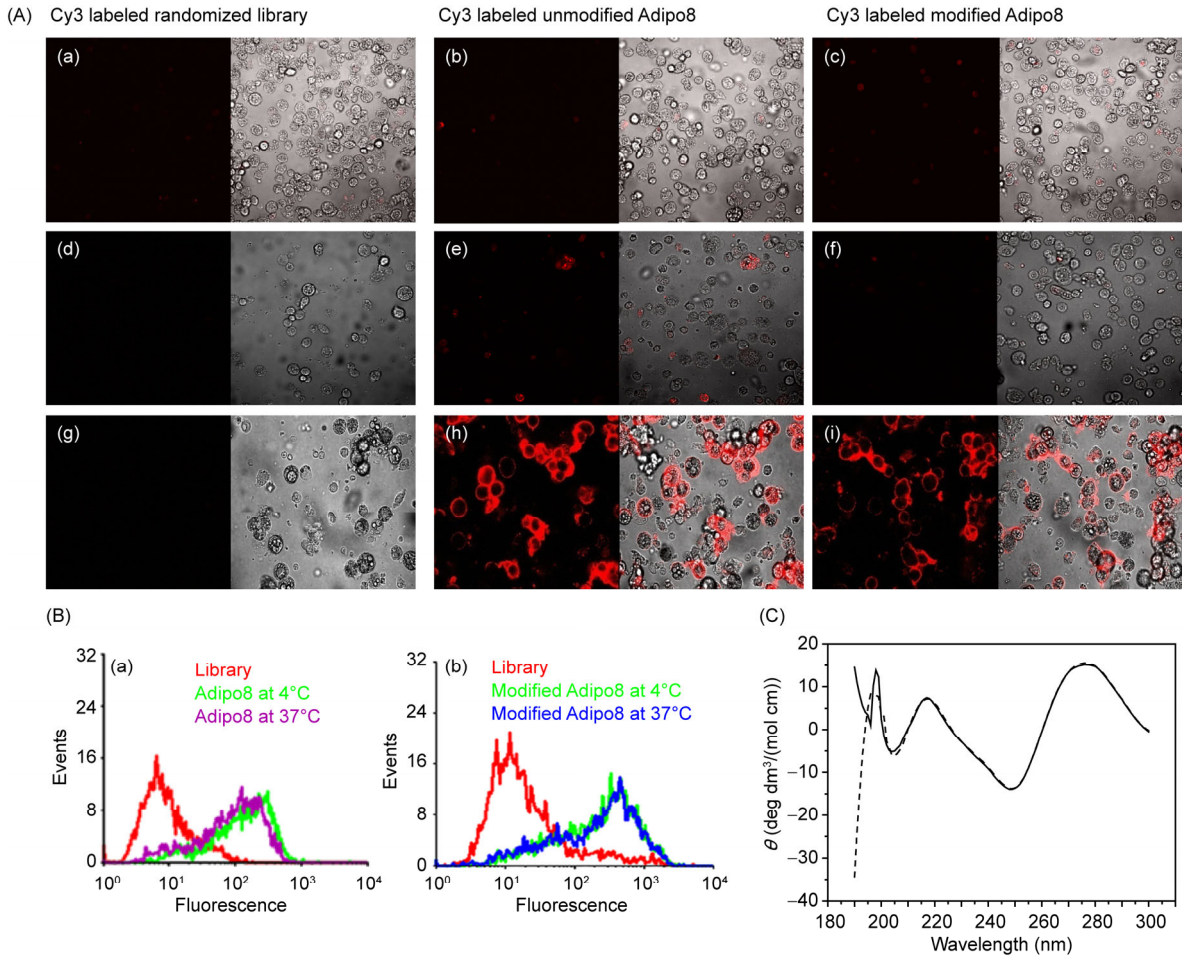

Figure 1 Confocal images of random library, unmodified, and PS-modified Adipo8 staining of cultured differentiated adipocytes. (A) The mature brown adipocytes (a, b, c), 3T3-L1 preadipocytes (d, e, f), and differentiated mature adipocytes (g, h, i) were each incubated separately with a randomized library, unmodified Adipo8, or PS-modified Adipo8 labeled with Cy3, and the binding affinity of each aptamer was evaluated by confocal imaging; (B) flow cytometry assays to assess whether the binding ability of Adipo8 (a) or PS-modified Adipo8 (b) to mature adipocytes was influenced by temperature; (C) CD spectra of 10 μmol/L PS-modified Adipo8 in PBS, pH 7.2. Spectra were measured at 4 (solid line) and 37 °C (dotted line).

modified Adipo8 was tested. To accomplish this, unmodified and modified aptamers were separately incubated with target cells at 4 and 37 °C, and no differences in the binding ability of either aptamer were detected at either temperature (Figure 1(B)), revealing that the high binding ability of these aptamers was preserved at 37 °C in addition to 4 °C. Thus, the Adipo8 aptamer could potentially be used for *in vivo* applications.

Structural studies using circular dichroism (CD) spectroscopy can provide reliable information for identifying aptamer structures. If a conformation changes with temperature, the results obtained above might not be representative of those obtained in *in vitro* assays. Positive maximal peaks were observed at 220 and 280 nm, and a negative minimal peak occurred at 250 nm (Figure 1(C)). Based on a previous report, the absence of change in CD spectra between 4 and 37 °C confirms the stability of the secondary conformation of the aptamer at different temperatures. These results preliminary indicate that this modification does not interfere with the robust binding capacity of Adipo8.

Additionally, the PS-modified Adipo8 binding process during the adipogenic differentiation of 3T3-L1 cells was assessed. These aptamers were incubated with differentiating 3T3-L1 preadipocytes and were harvested at 0, 2, 4, 6, 8 or 10 days during the differentiation period. The fluorescence intensity was then monitored using confocal fluorescence microscopy to assess the binding affinity of this modified aptamer at these time points. Based on confocal imaging (Figure 2), very little fluorescence signal was detected from adipocytes on days 0 and day after the induction of differentiation. On day 4, a weak fluorescent signal could be observed in partially differentiated adipocytes, after which the binding affinity stabilized and even increased. On day 10, a strong and specifically bound fluorescent signal could be observed in differentiated adipocytes. The binding pattern coincided with our former study [14]. This finding confirmed that Adipo8 had no binding affinity with 3T3-L1 preadipocytes, but that an increased and more specifically bound fluorescent signal could be easily observed upon differentiation of 3T3-L1 preadipocytes, suggesting that this kinetic specificity could have therapeutic value in the context of drug intervention or delivery.

3.2 Stability of PS-modified Adipo8 against nucleases in serum-containing medium

Single-stranded regions of nucleic acids are the primary targets of nuclease attack. Thus, conformational flexibility can lead to increased aptamer accessibility to nucleolytic degradation, making the reduction of such flexibility a key prerequisite for the success of an aptamer in *in vivo* applications. To this end, the incorporation of PS linkages can prolong the half-life of aptamers circulating in cellular and serum fluids [27]. Herein, the sensitivity of unmodified and PS-modified Adipo8 to serum nucleases was measured using an *in vitro* nuclease stability assay. Both aptamers were incubated with 90% human serum at 37 °C for up to 24 h, and samples were analyzed at different time points by denaturing PAGE. After running denaturing polyacrylamide gel electrophoresis (PAGE), band densities were measured

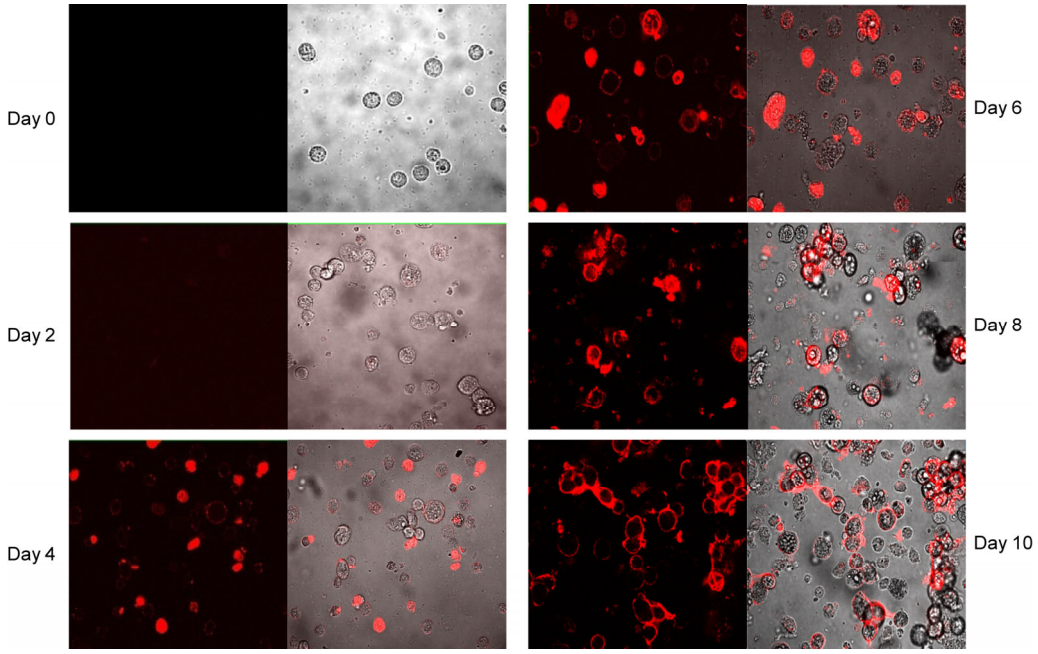

Figure 2 Analysis of aptamers staining of differentiated 3T3-L1 adipocytes by confocal microscopy. Modified Cy3-labeled Adipo8 was administered at a final concentration of 250 nmol/L in binding buffer. Images show 3T3-L1 preadipocytes at different time points during their differentiation into mature white adipocytes; fluorescence (left) and confocal (right) images are shown.

to define the percentage of intact aptamer. We found that unmodified Adipo8 was degraded by 30% within 12 h and by 40% within 24 h (Figure 3). By contrast, the PS-modified Adipo8 displayed remarkable stability. Compared with the unmodified aptamer, PS-modified Adipo8 showed significantly increased stability, especially at 12 and 24 h (*P<0.05). These data indicated that the modification of Adipo8 with a PS-linkage conjugated to PEG could prolong the stability of Adipo8 by preventing exonuclease attack. Therefore, with proper modifications, the aptamers could be beneficial when used for *in vivo* interventions and even in obesity drug delivery.

Unmodified and PS-modified aptamers were separately incubated with 10% FBS media at 37 °C and samples were analyzed at different time points (0, 1, 2, 6, 12 and 24 h); the percentage of intact aptamers was defined by measuring the band density after running denaturing PAGE (*P<0.05).

3.3 Specific binding of PS-modified Adipo8 to adipose tissue

Adipo8 was obtained using cell-SELEX, as described above.

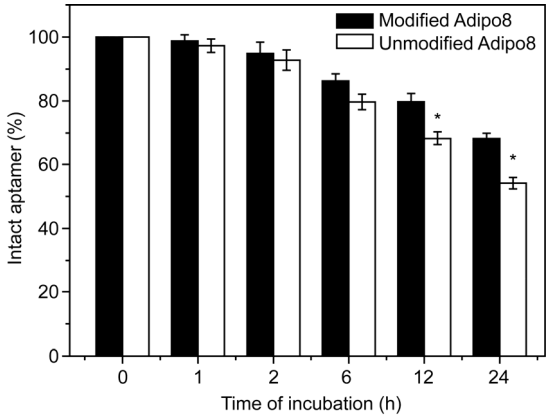

Figure 3 Nuclease resistance exhibited by Adipo8 in DMEM with 10% FBS.

Although preliminary data to test its binding specificity and affinity to mature white adipocytes was collected from *in vitro* assays, it remained necessary to test these features in clinical samples to assess its potential for use in targeted drug delivery. Aptamers are introduced *in vivo* by various intravenous routes, among which intraperitoneal (i.p.) injection is the most convenient and allows most drugs to be quickly absorbed [28]. To investigate the specific binding of Adipo8 with adipose tissue *in vivo*, PS-modified Adipo8, which shows more stability in complex biological matrixes, was adopted and labeled with Cy3. The Cy3-labeled PS-modified Adipo8 was then injected i.p. into DIO mice, which were evaluated by H&E staining and fluorescence imaging (Figure 4). A strong fluorescent signal could be observed in adipose tissue, whereas only a weak signal was detected in the liver, skeletal muscle and renal tissues by upright fluorescence microscopy. These results verified the high affinity and specificity of Adipo8 binding to WAT *in vivo*, similar to *in vitro* testing results described above, which indicated that PS-modified Adipo8 is a tissue-specific tool with potential uses in targeted drug delivery for the treatment of obesity.

3.4 Analysis of PS-modified Adipo8 degradation *in vivo*

Unmodified aptamers can be easily degraded by nuclease digestion, and the half-life is correspondingly short. However, PS-modified bases reduce nuclease decomposition, but also decrease the melting tempreture (T_m) value. To eliminate these effects, partial bases at both ends of the primers were selected for phosphorothioate modifications. The low molecular weight of aptamers has limited their clinical application because of fast renal clearance. However, the molecular weight of aptamers can be increased by conjugation to PEG or cholesterol, which can delay renal clearance. To compare the effects of such modifications on Adipo8 stablity *in vivo*, DIO mice were injected i.p. with either

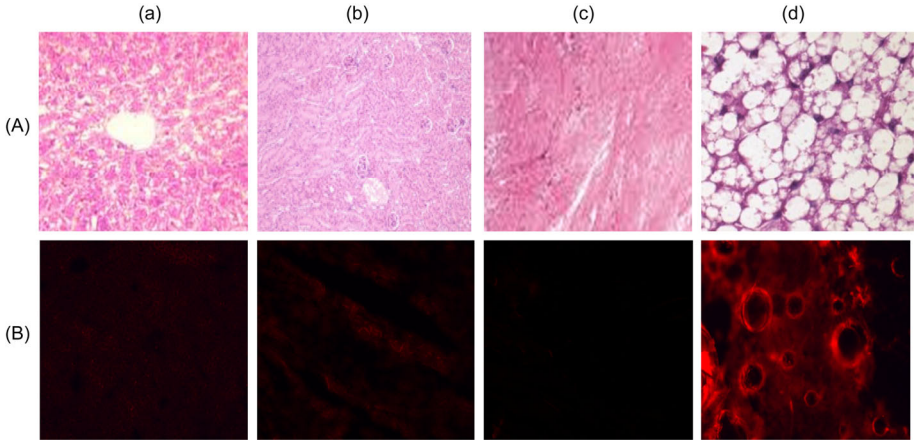

Figure 4 Specific binding of PS-modified Adipo8 to adipose tissue. (A) H&E staining of hepatic tissue (a), renal tissue (b), skeletal muscle tissue (c), and epididymal adipose tissue (d) from DIO mice that were euthanized 1 h post-intraperitoneal injection; (B) fluorescence imaging of the Cy3 labeled tissues.

modified or unmodified Adipo8. Mice were sacrificed 24 h later, and epididymal adipose tissue was removed, followed by testing for fluorescent signals by fluorescent microscopy (Figure 5). Compared with the PS-modified Adipo8 group, adipose tissue in the unmodified Adipo8 group showed a much weaker fluorescence signal at each testing time. Moreover, loss of fluorescence signal in the unmodified Adipo8 group resulting from degradation increased over time. Nevertheless, a very strong fluorescence signal could be observed 1 h after injection in the PS-modified Adipo8 group. Degradation of the fluorescent signal in the PS-modified Adipo8 group was reduced compared to the unmodified Adipo8 group. At 24 h after injection, the fluorescent signal could still be detected. These findings demonstrated that Adipo8 modification could increase biostability and prolong the *in vivo* binding time. Therefore, this modification provides an additional indication that nuclease decomposition can be reduced and that renal clearance of aptamers can be delayed [29,30]. This delayed renal clearance affects pharmacodynamics, as it results in increased concentrations in serum and prolonged drug action time. The prolonged binding time might allow elastic adjustments to the concentration and action time of aptamer-drug compounds to be made in order to target adipose tissues. To some extent, it demonstrated potential convenience in targeted drug delivery for the treatment of obesity.

3.5 PS-modified Adipo8 inhibits intracellular lipid accumulation and reduced PPAR-γ expression in adipocytes

As functional DNA molecules hold great promise for future applications in nanotechnology and bioanalysis, aptamers are known for their specific binding to target-specific ligands. Accordingly, they are regarded as potential candidates for including in sensing, imaging, and even therapeutic approaches [31–33]. It is generally believed that the accumulation of excess mature white adipocytes plays a dominant role in the progression of obesity. To observe the effect of PS-modified Adipo8 on intracellular lipid accumulation throughout adipocyte differentiation, 3T3-L1 cells were treated with randomized sequences and PS-modified Adipo8 during the differentiation process. We differentiated 3T3-L1 preadipocytes as described previously. PS-modified Adipo8 and randomized control sequences at a concentration of 250 nmol/L were added to the differentiation media beginning on day 4, 6, or 8, a blank control group was also included. On day 6 after differentiation, lipid droplets could be easily detected in the cytoplasm of the blank group cells. As differentiation progressed, the adipocytes size increased and the lipid droplets enlarged. The accumulation of lipid droplets was observed in the cytoplasm of 90% of adipocytes under an inverted microscope from days 8 to 10. Few difference could be observed between the randomized sequence control group and the blank control group based on droplets stained with Oil Red O. On day 6, the PS-modified Adipo8 group showed little significant difference from control groups. However, on day 8 and 10, compared with blank and randomized sequence control groups, lipid droplets in the PS-modified Adipo8 group became smaller, the size of adipocytes had also shrunk based on a visual observation (Figure 6). Compared with control cells, we observed a slight trend for Adipo8 to enhance the inhibition of lipid accumulation, a phenomenon observed from day 8 throughout differentiation. The differentiation and apoptosis of white adipocytes is well known to affect cell abundance, while changes in size depend upon the balance of adipogenesis and lipolysis activities. We observed a slight trend for Adipo8 to inhibit lipid accumulation, but this occurred not by reducing cell abundance but rather by reducing the cell size. Thus, the mechanism underlying the inhibition of intracellular lipid accumulation in white adipocytes by Adipo 8 remains to be fully elucidated.

Fat accumulation is often coupled with the activation of specific signaling molecules, such as PPAR-γ, a well-known adipogenic marker that shows increased expression levels

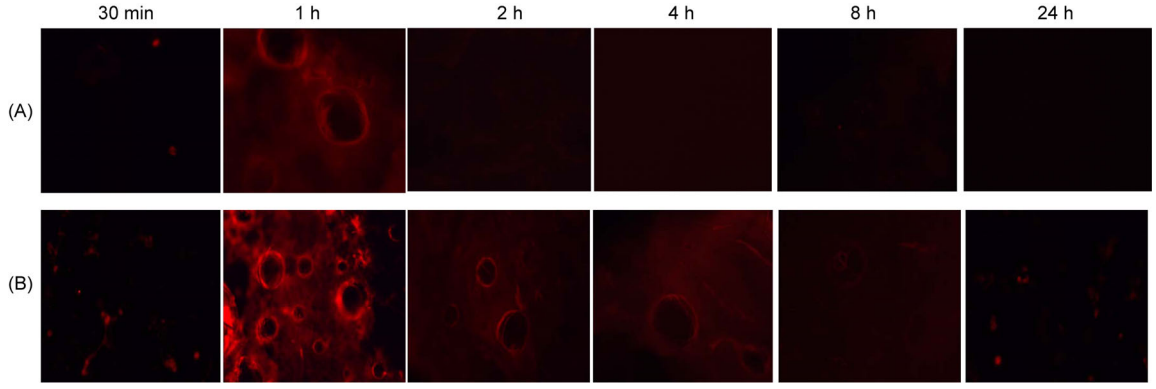

Figure 5 Monitoring PS-modified Adipo8 degradation *in vivo*. Fluorescence imaging of Cy3-labeled tissues. (A) Unmodified Adipo8 was injected i.p. into DIO mice, and the fluorescent signal was detected by fluorescence microscopy; (B) PS-modified Adipo8 was injected i.p. into DIO mice, and the fluorescent signal was measured.

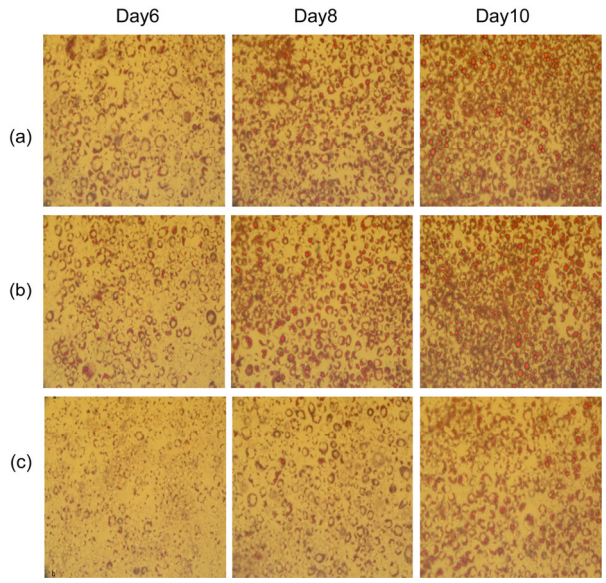

Figure 6 Adipo8 inhibits intracellular lipid accumulation in adipocytes. Cultured adipocytes were stained with Oil Red O. (a) Blank control group; (b) randomized sequence control group; (c) PS-modified Adipo8 group.

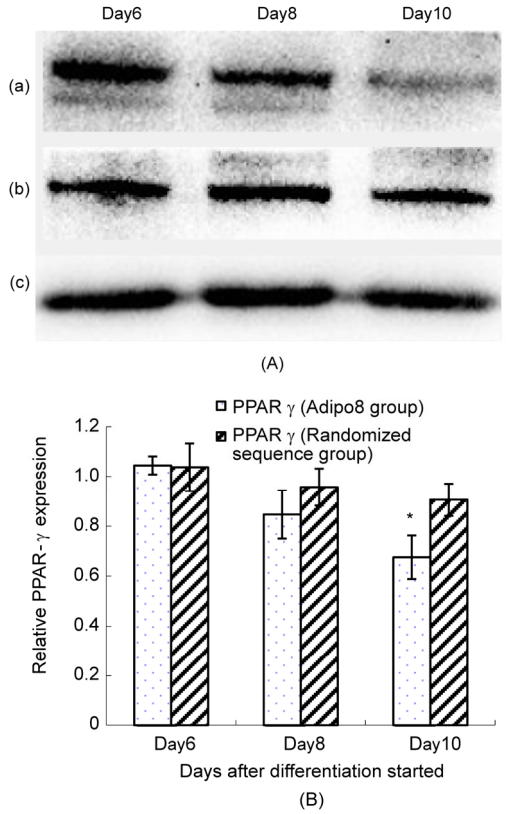

Figure 7 (a) PPAR-γ expression in adipocytes treated with adipo8; (b) PPAR-γ expression in adipocytes treated with randomized sequence groups; (c) GAPDH expression in adipocytes.

during adipocyte differentiation. As a key regulator of adipocyte biology, PPAR-γ induces adipogenic gene expression, facilitates adipose remodeling, and promotes lipid storage and energy homeostasis in adipocytes. It has been reported that PPAR-γ is positively correlated with the efficient adipogenesis of white adipocytes [34]. Therefore, to investigate whether reduced lipid accumulation in Adipo8-treated adipocytes results from the inhibition of adipocyte differentiation, we measured the PPAR-γ expression. On day 6 and 8, no significant differences ($P>0.05$) were observed in the randomized sequence or PS-modified Adipo8 groups (Figure 7). However, on day 10, PPAR-γ expression was reduced in the PS-modified Adipo8 group compared with the randomized sequence group ($*P<0.05$). We performed preliminary tests of the mechanism of Adipo8 intervention in the adipogenic differentiation of white adipocyte by downregulating PPAR-γ expression, but more testing should be conducted to assess signaling molecules that are upstream or downstream of PPAR-γ. Additionally, the kinetics of the activation of various signal pathways should be studied to ascertain the detailed mechanism underlying the effects of Adipo8 on PPAR-γ [35].

4 Conclusions

This study confirmed the ability of a modified Adipo8 aptamer to distinguish mature white adipocytes from either 3T3-L1 preadipocytes or brown adipocytes *in vitro*. PS-modified Adipo8 also showed enhanced biostability and prolonged binding time in biological fluids. Adipo8 could inhibit adipogenic differentiation of adipose tissue, possibly through the inhibition of PPAR-γ in adipose tissues.

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