Effect of a Nanoparticle-Based Multivalent Targeted Proapoptotic Peptide on Obesity

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ABSTRACT SUMMARY
Antiangiogenesis has been the focus of a new strategy for the treatment of obesity. Compared to the use of a peptidomimetic (Adipotide), a proapoptotic peptide encapsulated in prohibitin (an adipose vascular marker)-targeted nanoparticles (PTNP) caused a significant reduction in body weight in parallel with serum leptin, ectopic fat deposition in liver and muscle in diet-induced obese mice. Notably, the potential activity of PTNP was mediated via multivalent active recognition and uptake by primary adipose endothelial cells and the subsequent enhanced delivery into cytosol to be escaped from endosomes.

INTRODUCTION
The growth of adipose tissue is dependent on angiogenesis1. For targeting adipose vessels, Kolonin, MG et al. reported a small peptide (KGGRAKD) that could specifically bind to endothelial cell-surface prohibitin in white fat vessels (WFV) and was subsequently fused with a cell death-inducing peptide [D(KLAKLAK)2, KLA]. The s.c injection of the peptidomimetic CKGGRKDC-GG(D(KLAKLAK)2, termed as Adipotide, was reported to promote weight loss in obese animals2. Thus, adipose vascular disruption by antiangiogenic therapeutics is a promising strategy in terms of inhibition/depletion of angiogenesis-dependent adipogenesis (obesity).

We recently developed a prohibitin-targeted nanoparticle (PTNP) whose surface was modified with a linear peptide containing a WFV-targeting motif (KGGRAKD), attached via a long polyethylene glycol (PEG) spacer and a short PEG-polymer as a surface biostabilizer. Systemically injected PTNP to DIO mice is potentially boosted by the passive accumulation in obese fat through the EPR-like mechanism and therefore, was loaded KLA into PTNP (KLA-PTNP) that had been shown a potential boon to antiobesity3. However, little is known about whether antiangiogenesis is advantageous or not to debug the co-morbidity of DIO and metabolic syndrome. Our goal is to develop an effective antiangiogenic nanotherapy for DIO and dysfunctional adipose tissue, a major mediator for metabolic syndrome that has been manifested by ectopic fat deposition.

EXPERIMENTAL METHODS
An around 100 nm KLA-PTNP comprising of egg yolk phosphatidylcholine and cholesterol was prepared by a previously described REV method3. DIO mice were subjected to an i.v. injection of 0.2 mmol/kg of empty-PTNP, 1.0 and 3.0 mg/kg of KLA-PTNP and Adipotide at every 3 day for 30 days whereas the other group remained untreated. After the treatment, mice were sacrificed, adipose, liver and muscle tissues were removed and was then separated serum from blood by centrifugation for 10 min at 1400 × g. Immunostaining of formalin-fixed tissue pieces was processed as described previously1. These slices were then stained with BODIPY, rhodamine-labeled phalloidin (Invitrogen) and Hoechst 33342 and were viewed under a confocal microscopy (CLSM). For the quantification of fluorescence of BODIPY, rhodamine-phalloidin and Hoechst33342, we used Image-Pro(4) Plus-4.5 software (Media Cybernetics). Leptin and adiponectin in serum were determined by ELISA assay (R&D systems).

Primary endothelial cells from murine adipose tissue (pcEC-IWAT) were isolated and pcEC-IWAT cells were prepared as described previously3, 4. Then, rhodamine-PTNP and rhodamine-ligand at a rhodamine dose (200 nM) were added and incubated for 1 h. Then, cells were washed 3 times with 1ml of heparin in PBS (40 units/ml) and treated with Reporter Lysis Buffer (Promega Corp.) followed by centrifugation at 12,000rpm for 5min at 4℃ to remove debris. The cellular uptake efficiency of the rhodamine-PTNP and ligand were determined by measuring the fluorescence intensity of rhodamine (excitation at 550nm and emission at 590 nm) using FP-750 Spectro-fluorometer (JAS Co, Japan). For endosomal escape study, pcEC-IWAT was incubated with rhodamine-PTNP and rhodamine-ligand for 3h, washed and stained with lysosensor green DND-189 and was observed by CLSM.

RESULTS AND DISCUSSION
Obesity relies on a couple processes of angiogenesis and adipogenesis4. To apply PTNP for weight loss, KLA was encapsulated in PTNP (KLA-PTNP) and Adipotide as illustrated in Figure 1A. After intravenous injection of these preparations into DIO mice, the weight gain of NT and E-PTNP mice was accelerated by HFD feeding, whereas weight gain for both Adipotide and KLA-PTNP mice was significantly less than that of NT group (Fig 2B). On day 30 of the treatment, body weight of KLA-PTNP mice was significantly less than their initial weight (14% reduction), whereas that of Adipotide treated mice was not (5% reduction). It has been reported that serum leptin levels are correlated with the percentage of body fat whereas that of adiponectin is reduced in obese animals5, 6. Treatment of mice with Adipotide showed a
Therefore, the findings of obesity therapy should be escaped from endosomes.

Collectively, these data clearly demonstrate that the higher therapeutic effects of targeted nanotherapy are mediated by the multivalent targeting activity of PTNP to pcEC-IWAT cells and enhanced antiobesity by increased delivery of drugs into cytosol to be escaped from endosomes.

CONCLUSION

In summary, the findings presented herein show that KLA-PTNP has the potential to specifically deliver therapeutic concentrations of drugs to WFV and therefore, are potentially reversing obesity and ectopic fat storage in the liver and muscle of DIO mice, compared to the chimeric peptide. The potential activity of PTNP might be mediated, not only by its multivalent active targeting, but also the enhancement of drug delivery into the cytosol to be escaped from endosomes.

REFERENCES

1. Nishimura, S.; et al., 2007, Diabetes, 56 (6), 1517-1526.

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