Biomaterials 73 (2015) 32–41

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Enhanced thermogenic program by non-viral delivery of combinatory browning genes to treat diet-induced obesity in mice

Hongsuk Park [a], Sungpil Cho [b], Margit M. Janat-Amsbury [b], You Han Bae [c, d, *]

[a] Department of Bioengineering, University of Utah, Salt Lake City, UT 84112, USA
[b] Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, University of Utah, Salt Lake City, UT 84132, USA
[c] Department of Pharmaceutics and Pharmaceutical Chemistry, University of Utah, Salt Lake City, UT 84112, USA
[d] Utah-Inha Drug Delivery Systems and Advanced Therapeutics Research Center, Incheon, Republic of Korea

ARTICLE INFO

Article history:
Received 21 May 2015
Received in revised form
4 September 2015
Accepted 9 September 2015
Available online 11 September 2015

Keywords:
Thermogenic program
Obesity
Dual browning gene construct
IAPP
FNDC5
Irisin

ABSTRACT

Thermogenic program (also known as browning) is a promising and attractive anti-obesity approach. Islet amyloid polypeptide (IAPP) and irisin have emerged as potential browning hormones that hold high potential to treat obesity. Here, we have constructed a dual browning gene system containing both IAPP and irisin (derived from fibronectin type III domain containing 5; FNDC5) combined with 2A and furin self-cleavage sites. Intraperitoneal administration of the construct complexed with a linear polyethylenimine into diet-induced obese mice demonstrated the elevation of anti-obesogenic effects characterized as the decreased body weight, adiposity, and levels of glucose and insulin. In addition, the construct delivery increased energy expenditure and the expression of core molecular determinants associated with browning. The additional advantages of the dual browning gene construct delivery compared to both single gene construct delivery and dual peptide delivery can be emphasized on efficacy and practicability. Hence, we have concluded that dual browning gene delivery makes it therapeutically attractive for diet-induced obesity treatment.

1. Introduction

Obesity has been viewed as a serious health care issue on an epidemic scale, because sedentary living styles and the consumption of calorie-dense food have been prevalent in developed and developing countries [1,2]. Such an epidemic movement of obesity throws alarming issues, since a host of obese people have been suffering from many non-communicable obesity-associated diseases, including heart disease, stroke, type 2 diabetes, and certain cancers [2,3]. Moreover, the prevalence of obesity not only causes economic burden but also staggers medical care expenses globally [4]. Along these lines, there is an emergent need for efficacious and pragmatic anti-obesity treatments.

Thermogenic program (also known as browning and brown fat development) is the biological conversion of white adipocytes into beige/brite adipocytes in response to various stimuli [2,5]. In terms of anti-obesogenic effects, the expansion of beige/brite fat in subcutaneous inguinal white adipose tissues (IngWATs) has been suggested as a potential therapeutic approach to obesity treatment, because the conversion can raise energy expenditure [6]. There are three key molecular determinants of thermogenic program, which are peroxisome proliferator-activated receptor γ co-activator 1α (Ppargc1α), PR domain containing 16 (Prdm16), and peroxisome proliferator-activated receptor γ (Pparγ) [5]. Ppargc1α is a transcriptional regulator with respect to energy metabolism, and it is essential for appropriate thermogenic program [7,8]. Prdm16 also stimulates thermogenic program, and its genetic overexpression escalates energy expenditure and ameliorates glucose tolerance in diet-induced obese (DIO) mice [9]. Pparγ facilitates the formation of beige/brite adipocytes, and it can inhibit the WAT-related genes [10].

Islet amyloid polypeptide (IAPP; also known as amylin) and irisin have garnered significant interests, because both of them can elicit thermogenic program [5]. IAPP is released from pancreas, and results in the increase in energy expenditure [11]. Irisin, recently identified from fibronectin type III domain containing 5 (FNDC5), has precipitated a steep rise in thermogenic program investigation due to its efficient conversion of WAT into brown adipose-like tissues, and improved glucose tolerance [6]. On the basis of these

* Corresponding author. Department of Pharmaceutics and Pharmaceutical Chemistry, University of Utah, Salt Lake City, UT 84112, USA. Tel.: +1 801 585 1518.
E-mail address: you.bae@utah.edu (Y.H. Bae).

http://dx.doi.org/10.1016/j.biomaterials.2015.09.011
0142-9612/

findings, IAPP and irisin can be promising therapeutic candidates for obesity management.

Since obesity is a complex condition caused from a diverse of factors, combinatorial hormone therapies have been attracted for successful obesity treatment [12]. In order to demonstrate its efficacy, the dual peptidergic therapies have been tried, and they appeared to be a therapeutically attractive way to intervene obesity. However, they need frequent administrations due to the proteins' short half-life, and they demand high costs, and even trigger harmful immune responses [13–15]. To break through these limitations, the development of dual gene construct can be suggested, because it can achieve the better patient compliance by less frequent injections and the cost-effective treatment without the preparation of therapeutic peptides in a scale. As noted above, IAPP and irisin can be potential therapeutic agents for anti-obesity, and further, a combination of both genes can be considered as a desirable approach to effective obesity management. Thus, we conducted the development of dual browning gene construct containing both IAPP and irisin, and its delivery method, assuming that it provides more effective and practical anti-obesity treatment.

The dual browning gene construct can be prepared using 2A and furin self-cleavage peptide linker genes between IAPP and FNDC5 (the origin of irisin) [6] genes in a single mammalian plasmid. The 2A system allows different genes to express their matching proteins discretely in equimolar concentrations in cell type-independent manner. This is a versatile site when the independent expression of individual proteins are required [16–18]. Among 2A peptide family, porcine teschovirus-1 2A (P2A) shows the highest cleavage ability in mammalian cells [19]. Moreover, furin cleavage site aids the higher and more controlled protein expression without possible adverse effects from the residual 2A peptides [20]. Hence, we inserted furin and P2A (FP2A) linker gene between IAPP and FNDC5 genes to establish a dual browning gene construct.

Polymers-based delivery system has been regarded as the useful strategy, given that its diverse modifications, low immunogenicity and toxicity [21,22]. In particular, linear polyethylenimine (lPEI) with a molecular weight of 25 kDa has been widely harnessed for gene delivery due to its effective gene transduction by endosomolysis in intracellular milieu, and low cytotoxicity. This is why it is regarded as a gold standard for polymer-based gene delivery [23–26]. Specifically, for *in vivo* applications, the N:P ratio (the mole ratio of cationic amines from polymers to anionic nucleic acid phosphates from genes) of 5, in which lPEI is complexed with plasmid DNA (pDNA), is appropriate, because the ratio has outstanding stability against DNase, and optimal transfection efficiency and toxicity balance [18,27–30].

Previously, our group proved that the combinatorial gene construct containing IAPP and leptin genes delivered by lPEI exhibited a synergistic effect on body weight loss in DIO mice [18]. In the current work, we have revealed that intraperitoneal (i.p.) administration of the combinatorial gene construct with a pair of browning genes, IAPP and irisin, delivered by lPEI led to the augmentation of anti-obesogenic thermogenic program in DIO mice, illustrating the increase in energy expenditure. Furthermore, we have also verified that this therapeutic system elevated the three key browning gene (Ppargc1α, Prdm16, and Pparγ) expressions (Fig. 1A). To our best knowledge, this is the first report to highlight that the two browning gene combination delivery has shown its promise on the treatment of obesity.

2. Materials and methods

2.1. Materials

Maxiprep kits were purchased from Macherey–Nagel (Bethlehem, PA, USA). In-Fusion™ Kits, Xfect™ Transfection Reagent, and TaKaRa BioMasher Standard were bought from Clontech (Mountain View, CA, USA). pCR-blunt-TOPO-FNDC5 was purchased from Addgene (Cambridge, MA, USA), and IAPP Mouse cDNA Clone was bought from Origene (Rockville, MD, USA). Restriction enzymes, EcoRI, NotI, BamHI, and HindIII, M-MuLV Enzyme Mix, M-MuLV Reaction Mix, and OneTaq® RT-PCR Kit were obtained from New England Biolabs (Ipswich, MA, USA). lPEI (weight average molecular weight 25 kDa) was purchased from Polysciences (Warrington, PA, USA). pcDNA3-complementary T7 and SP6 primers were obtained from University of Utah Core Facility (Salt Lake City, UT, USA). pcDNA3, Trizol, quantitative reverse transcription polymerase chain reaction (qRT-PCR) kit, Eukaryotic 18S rRNA Endogenous Control, and Single Tube TaqMan® Gene Expression Assays were bought from Invitrogen (Carlsbad, CA, USA). RNA Clean & Concentrator™-5 was obtained from Zymo Research (Irvine, CA, USA). Human Embryonic Kidney (HEK) 293T cell was purchased from ATCC (Manassas, VA, USA). RNeasy Mini Kit was bought from Qiagen (Valencia, CA, USA). IAPP and Irisin EIA Kits were obtained from Phoenix Pharmaceuticals (Burlingame, CA, USA). Mouse Insulin ELISA Kit, FFA test Kit, and TG assay solutions were purchased from Crystal Chem, Roche, and Wako Diagnostics (Richmond, VA, USA), respectively.

2.2. Plasmid construction

Recombinant pDNAs, monocistronic pcDNA3-IAPP and pcDNA3-FNDC5, and bicistronic pcDNA3-FP2A-IAPP-FNDC5, were prepared using mouse-originated IAPP and FNDC5 open reading frames (ORFs). As for the bicistronic plasmid construction, since FNDC5 ORF has an amount of GC content, it is hard to PCR-amplify the FNDC5 gene [31]. Thus, to make the bicistronic pcDNA3-FP2A-IAPP-FNDC5, we first obtained FNDC5 gene from pCR-blunt-TOPO-FNDC5 by double enzyme cut with BamHI and NotI, and ligated into pcDNA3, resulting in pcDNA3-FNDC5 production (The monocistronic pcDNA3-FNDC5 was constructed). After that, we designed forward and reverse PCR primers including more than 15 base pair overlap with the neighboring segment of pcDNA3-FNDC5. The primers also contain translation initiation site (Kozak), restriction enzyme sites, and FP2A linker gene. In addition, they have more than 20 base pairs of IAPP ORF-complementary sequences. Next, we performed PCR with the primers and IAPP cDNA plasmid, followed by the ligation of PCR amplicon with linearized pcDNA3-FNDC5 using In-Fusion™ reaction to complete pcDNA3-FP2A-IAPP-FNDC5 preparation [32]. The primer sequences for pcDNA3-FP2A-IAPP-FNDC5 are provided (Table S1). For pcDNA3-IAPP construction, IAPP ORF gene was obtained from IAPP cDNA plasmid by double enzyme cut with EcoRI and NotI, followed by sub-cloning of the IAPP gene into digested pcDNA3.

2.3. Confirmation of target gene inserts and gene expression of plasmids by gel electrophoresis

To determine the correct construction of three newly developed plasmids, pcDNA3-IAPP, pcDNA3-FNDC5, and pcDNA3-FP2A-IAPP-FNDC5, gel electrophoresis was monitored after incubation with the corresponding restriction enzymes. To check into the gene expression from each plasmid, they were introduced into HEK293T cells using Xfect™ Transfection Reagent according to the manufacturer's instruction. The HEK293T cells in all the experiments of this study were cultured in DMEM bought from ATCC (Manassas, VA, USA), supplemented with 10% fetal bovine serum in 5% CO_2 humidified atmosphere at 37 °C. Briefly, HEK293T cells were cultured in six-well plate at initial density of 5×10^5 per well for one day. Then, 5 μg of pDNA complexed with 1.5 μl of Xfect™ was