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Gene expression profiles of adipose tissue of obese rats after central administration of neuropeptide Y-Y5 receptor antisense oligodeoxynucleotides by cDNA microarrays

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ABSTRACT

To investigate the gene expression profiles of adipose tissue of obese rats after central administration of neuropeptide Y-Y5 receptor antisense oligodeoxynucleotides (ODNs), Y5 receptor antisense, mismatched ODNs or vehicle was intracerebroventricularly injected and cDNA microarrays were undertaken. Central administration of NPY-Y5 receptor antisense ODNs decreased food intake, body weight and serum insulin compared with both vehicle and mismatched ODNs. The average area of adipocytes both at retroperitoneal and epididymal adipose tissue were fall in antisense group while only the weight of the retroperitoneal fat pats was reduced in antisense group. cDNA microarrays containing 18,000 genes/Ests were used to investigate gene expression of adipose tissue. Autoradiographic analysis showed that 404, 81, and 34 genes were differently expressed over twofold, threefold, and fivefold, respectively. The analysis of gene expression profiles indicated that 332 genes were up-regulated and 187 genes were down-regulated in response to Y5 receptor antisense ODNs treatment. Different clusters of genes associated with apoptosis, signal transduction, energy metabolism, lipid metabolism, etc., such as FXR1, PHLDA1, MAEA, PIK3R1, ICAM2, PITPN, CALM2, CAMK2D, PKIA, DRD2, SLC25A14, CKB, AADAC, LIPA, ACOX3, FADS1, were concerned. Analysis of differentially expressed genes will help to understand the effects of Y5 receptor antisense ODNs therapy.

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1. Introduction

Neuoropeptide Y (NPY), a 36-amino-acid neuromodulator abundantly expressed in the hypothalamus, has been implicated in the regulation of food intake and body weight. The effects of NPY are mediated by distinct receptor subtypes. NPY-Y1 and NPY-Y5 have become recognized as the most likely candidates for the mediation of the effects of NPY on

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feeding and energy expenditure [16,32]. In particular, the importance of NPY-Y5 has been suggested by the parallel pharmacology of the receptor in vitro and feeding in rodents [3,11,15], and the inhibitory effects of NPY-Y5 receptor antisense ODNs [4,30,31], NPY-Y5 deficiency [23], and an NPY-Y5 antagonist [6,17,22] on NPY-induced feeding and energy expenditure in rodents. But the molecular mechanisms of these effects have not been fully understood.

ODN technology appeared to be one of viable tools to characterize the physiological actions of NPY. The two best known physiological effects of NPY, namely, stimulation of feeding and enhanced secretion of reproductive hormones, have been characterized with the use of antisense ODNs [20]. Centrally administered antisense ODNs effectively blocked the effects of NPY, thereby demonstrating that neuronal permeability, degradation, and toxicity of ODNs are not limiting factors after icv administration [20]. Thus, ODN is also one of choices to study the function of Y5 receptor in vivo. Otherwise, with the advent of gene expression profile approaches, such as cDNA/oligo microarrays, suppression subtractive hybridization (SSH), and differential display reverse transcriptase polymerase chain reaction (DDRT-PCR), it has become possible to analyze thousands of genes simultaneously. For this reason, gene expression profiling provides an ideal tool for the global survey and identification of new players in complex pathophysiological processes.

So, in the present work, phosphothioate end-protected antisense ODNs targeted to the initiation codon of Y5 receptor mRNA was intracerebroventricularly injected. Food intake, body weight and serum insulin were monitored every 24 h during the following 2 days and average adipocyte area was calculated. Then we screened and identified differentially expressed genes in omental adipose tissue of obese rats after Y5 receptor antisense ODNs therapy by using cDNA microarrays. We focused on omental adipose tissue because it has been shown that fat from this location is more closely involved in the co-morbidities associated to obesity than fat from the subcutaneous depot [24]. Our data provided novel information for the identification of genes that may be implicated in the mechanisms of body weight loss by central administration of Y5 receptor antisense ODNs.

2. Materials and methods

2.1. Animals and diet

Forty-eight male Sprague–Dawley rats (4 weeks old) were obtained from Animal Center of Jiangsu Province. All rats were housed individually at 21–23 °C, in 12 h light/dark cycles and had a free access to food and water. A week later, these rats were assigned randomly into a group fed with standard rat chow (50% carbohydrate, 19% protein, 12% water, 4% fat and 2.1 kcal/g) ($n = 12$, weight: 77.91 ± 11.50 g), and a group fed with high-fat diet (34% carbohydrate, 17% protein, 4% water, 35% fat and 5.2 kcal/g) as a diet-induced obesity model, modified from other reports [9,27] ($n = 36$, weight: 77.82 ± 9.24 g, $P > 0.05$). Seven weeks later, the weights of obese models (362.92 ± 39.65 g) were significantly higher than those of normal control rats (315.22 ± 42.30 g, $P < 0.01$), which confirmed the successful preparation of obese models.

2.2. Surgery

Under sodium pentobarbital (50 mg/kg) anesthesia, 36 obese rats were stereotaxically implanted with stainless steel guide cannulas extending 3.75 mm below the external surface of the skull. The cannula was placed into the anterior horn of the lateral ventricle through a trepanation located 1 mm lateral to the bregma. After cannulation, the rats were individually housed in metabolic cages, allowing precise measurements of food intake. The animals were allowed a 7-day recovery period during which they were handled daily to habituate them to the injection procedure.

2.3. ODNs

Phosphothioate end-protected ODNs were obtained from Shanghai BioAsia Bio Technology Co, Ltd. The NPY-Y5 receptor antisense ODNs spanned 20 bp downstream from the start codon with the following sequence 5-GTG GAA GAA GAG GAC GTC CAT-3 [31]. Based on this sequence the scrambled ODN was constructed using the same numbers of CTGA nucleotides in a random order.

2.4. Injection paradigm

Before experiments were started, rats were randomized into three groups which received antisense ODNs, mismatched ODNs or vehicle, respectively. The rats were injected thrice daily (10:00 a.m., 1:00 p.m., 4:00 p.m.) for 2 days with 50 μg ODNs in 10 μl PBS. Food intake and body weight were monitored every 24 h during the following 2 days. On the third day (after a total six injections) rats were decapitated. Omental adipose tissue was stored under −70 °C. Trunk blood was also collected. Blood samples were then stored at −70 °C until analysis.

2.5. Measurement of the level of serum insulin

After 15 min of centrifugation at $1500 \times g$, the serum was transferred to polypropylene tubes and then frozen at −42 °C until insulin was assayed. Serum insulin was determined by radioimmunoassay (Huaying Bio-tech Institution, Beijing, China).

2.6. Morphological evaluation of white adipocytes

Serial slices of adipose tissue were prepared from rats in each group and stained with hematoxyline-eosin. Morphological evaluation was performed in five optical fields randomly taken in each slide at 200× magnification using MPIAS (multimedia pathological image & word analysis system)-500 software.

2.7. Total RNA isolation

Total RNA was extracted with Trizol reagent (Invitrogen, San Diego, CA, USA) using the procedure outlined in the manufacturer's protocol. RNA extracts from eight rats of each

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