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Effect of leptin on expressions of leptin receptors mRNA in HepG2 cells

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Abstract: Objective Leptin resistance is thought to be a main mechanism of human obesity. Although some studies suggested that leptin resistance could be relevant to the level of leptin receptor and its downstream signaling pathway, there has been little research on leptin receptor regulation. This paper studied the effect of leptin on its receptors. **Methods** The human hepatocellur carcinoma cell line HepG2 was incubated in serum2free medium containing 0, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6} M of human leptin respectively for 24 hrs. Then semi2quantitative RT2PCR was used to measure the changes of long (OB2Rb) and short (OB2Ra: OB2R219.1, OB2R219.3) leptin receptors mRNA expressions in HepG2 cells. **Results** Both OB2Ra and OB2Rb mRNA were expressed in HepG2 cells, which provided a useful model for studies of leptin receptors regulation. Leptin (10^{-7} - 10^{-6} M) induced a significant decrease in the OB2Rb mRNA expression, with the maximum effect at 10^{-6} M (0.43 ±0.14 vs 1.01 ±0.22), when compared with the control (incubation in the absence of leptin). Similarly, the expressions of OB2R219.1 and OB2R219.3, two isoforms of OB2Ra, were also markedly reduced in cells treated with 10^{-8} - 10^{-6} M leptin, with the maximum inhibition for OB2R219.1 at 10^{-7} M (44 % of the control) and for OB2R219.3 at 10^{-6} M (49 % of the control). **Conclusions** Leptin can inhibit the expressions of both OB2Ra and OB2Rb mRNA in HepG2 cells, which may be associated with leptin resistance in vivo.

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Key words: Leptin; Leptin receptor; HepG2 cell

瘦素对 HepG2 细胞瘦素受体 mRNA 表达的影响

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[**摘 要**] **目的** 瘦素抵抗被认为是单纯性肥胖儿童的主要发病机制。瘦素受体水平及其下游信号通路可能与瘦素抵抗有关。本实验研究瘦素对瘦素受体基因表达的影响,探讨瘦素抵抗的发生机制。**方法** 以 HepG2 细胞株为实验模型,利用细胞培养、DNA 序列测定及半定量的 RT2PCR 等方法,检测不同浓度(0,10^{-9},10^{-8},10^{-7},10^{-6}M)的瘦素对 HepG2 细胞瘦素短型受体(OB2Ra:OB2R219.1,OB2R219.3)及长型受体(OB2Rb)mRNA 的表达的影响。**结果** HepG2 细胞含有 OB2Ra 及 OB2Rb。当 HepG2 细胞与不同浓度的瘦素培养 24 h 后,10^{-7} - 10^{-6}M 浓度瘦素明显抑制了 OB2Rb 的 mRNA 表达,并在 10^{-6}M 浓度时作用最强(0.43 ±0.14 vs 1.01 ±0.22)。10^{-8}～10^{-6}M 浓度的瘦素亦明显抑制了 OB2R219.1 及 OB2R219.3 的 mRNA 表达,并在 10^{-7}和 10^{-6}M 浓度时分别达到最大抑制,为不含瘦素对照组的 44 %和 49 %。**结论** 瘦素对 HepG2 细胞瘦素受体表达有下调作用,这可能是体内瘦素抵抗的机制之一。

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[**关 键 词**] 瘦素;瘦素受体;HepG2 细胞

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Childhood obesity is becoming increasingly ap2 parent with the changes of children's life2styles, which is a risk factor for adult diseases such as type 2 diabetes and cardiovascular diseases. Leptin, coded by OB gene, is an adipocyte2derived hormone that plays a key role in the regulation of food intake, ener2 gy expenditure, and the body energy balance in ro2 dents and humans[1,2]. Leptin works through the leptin receptor (OB2R), two major isoforms of which are long form (OB2Rb) and short form (OB2Ra). Previous research has showed that the levels of leptin were high in obese children[3] and that the deficiency of leptin caused by gene mutation was not associated with obesity, which suggests that leptin resistance or insensitivity may be a leading cause of human obesity. Leptin receptors down2regulation induced by leptin it2 self may be a cause of leptin resistance[4,5]. Human hepatocellular carcinoma cell line (HepG2), derived from liver where both the OB2Ra and OB2Rb are ex2 pressed, was used in this research to investigate the effects of human leptin on OB2Rb and OB2Ra mRNA expressions for an understanding of the mechanism for leptin resistance.

Materials and methods

Cell cultures and treatments

The human hepatocellular carcinoma cell line HepG2 (American Type Culture Collection, USA) was cultured (5 % CO_2 at 37 ℃) in a 62well plate, 0.5×10^5 cells per well, using DMEM containing 10 % FBS, 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin and 0.25 μg/ml ampho2 tericin B. The culture medium was changed every other day. On the 6th day of culture, HepG2 cells were incubated in serum2free medium containing 0, 10^{-9}, 10^{-8}, 10^{-7} or 10^{-6} M recombinant human leptin (Diaclone Research, France) for 24 hours to analyze the effects of leptin on the expressions of OB2 Ra and OB2Rb.

Detection of leptin receptors mRNA expressions by RT2PCR

Total RNA was extracted from the treated cells using ISOGEN (Nippon Gene, Japan) according to the manufacturer's procedure. OD levels of RNA A260/A280 were measured and RNA concentrations were calculated. RNA (10 μg) was treated with 2 U of deoxyribonuclease (Nippon Gene, Japan) for 15 minutes at 37 ℃, then stored at - 70 ℃.

One μg RNA was used to synthesize cDNA with an Advantage RT2for2PCR kit (Clontech Inc., USA). PCR was performed using primers designed on the basis of established GenBank sequences for leptin re2 ceptors in a PTC2100 Programmable Thermal Controller (MJ Research Inc., USA). The human glyceraldehyde2 32phosphate dehydrogenase (GAPDH) was used as an in2 ternal control. To amplify leptin receptor mRNA, ini2 tial denaturation at 95 ℃ for 15 minutes was followed by 30 cycles of denaturation at 94 ℃ for 1 min, an2 nealing at a primer2specific temperature for 1 minute (Table 1) and extension at 72 ℃ for 2 minutes. The reactions were terminated by a final extension at 72 ℃ for 5 minutes. The PCR products were identified by sequencing in an ABI 373A Gene Scan (Applied Biosystems, USA). Ten microliter aliquots of PCR products were electrophoresed in 2 % agarose gels and stained with ethidium bromide. Video images of the ethidium bromide2stained gels were quantified by densitometry using the NIH ImageJ 1.61 software. A linear relationship between PCR products and am2 plification cycles (from 20 to 45) was observed.

Statistical analysis

Data were expressed as $x \pm s$. Statistical analy2 sis was performed by one2factor ANOVA and Fisher's LSD using the StatView 5.0 J program (Abacus Concepts, Inc. USA). A level of $P < 0.05$ was considered statistically significant.

Results

Expressions of OB2Rs isoforms in HepG2 cells

The expressions of human OB2R219.1, OB2 R219.3 and OB2Rb mRNA were demonstrated in HepG2 cells (Figure 1).

Effects of leptin on OB2Rs mRNA expressions

Leptin (10^{-7} - 10^{-6} M) induced a significant decrease in the OB2Rb mRNA expression, with a maximum effect at 10^{-6} M, when compared with the control (incubation in the absence of leptin). Similar2 ly, the expressions of two isoforms of OB2Ra, were

also markedly reduced in cells treated with 10^{-8} - 10^{-6} M leptin, with the maximum inhibition for OB2R219.1 at 10^{-7} M and for OB2R219.3 at 10^{-6} M (Table 2).

Table 1 Primer sequences, product length and primer2specific conditions for RT2PCR

Gene		Primer sequence (5'- 3')	Product length (bp)	Cycles	Annealing temperature (°C)
OB2Rb	forward	CAG AAG CCA GAA ACG TTT GAG	344	30	64
	reverse	AGC CCT TGT TCT TCA CCA GT			
OB2Ra					
OB2R219.1	forward	ATA GTT CCG AAC CCC AAG AAT	221	30	64
	reverse	CAA TAG TGG AGG GAG GGT CA			
OB2R219.3	forward	ATT CAA TTG GTG CTT CTG TT	573	30	62
	reverse	CAT TGG GTT CAT CTG TAG TG			

Figure 1 RT2PCR expressions of OB2Ra and OB2Rb mRNA in HepG2 cells

Note: A: OB2Rb. B: OB2R219.1. C: OB2R219.3. D: GAPDH. E: Negative controls. M: Molecular markers (1002bp ladder)

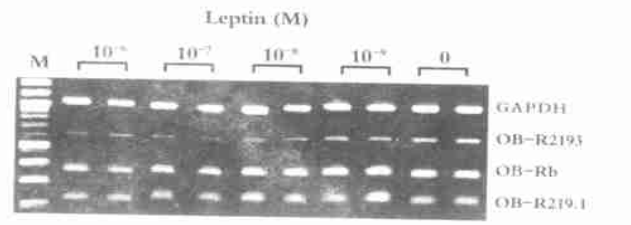

Figure 2 RT2PCR expressions of OB2Ra and OB2Rb mRNA in HepG2 cells treated with leptin

Table 2 Dose2dependent effect of leptin on OB2R mRNA levels in HepG2 cells ($n = 4$)

Leptin	0	10^{-9}M	10^{-8}M	10^{-7}M	10^{-6}M
OB2Rb	1.01 ±0.21	0.77 ±0.25	0.75 ±0.14	0.57 ±0.06[b]	0.43 ±0.14[b]
OB2R219.1	0.78 ±0.16	0.59 ±0.03	0.53 ±0.05[a]	0.35 ±0.06[b]	0.42 ±0.08[b]
OB2R219.3	1.02 ±0.11	0.94 ±0.18	0.72 ±0.12[b]	0.63 ±0.05[b]	0.51 ±0.31

Note: Compared with the control (incubation in the absence of leptin), a $P < 0.05$; b $P < 0.01$

Discussion

The OB2R is a single membrane2spanning recep2tor of the class I cytokine receptor family, consisting of extracellular, transmembrane and intracellular do2mains. The cloning of the leptin receptor gene has re2vealed that at least 5 different isoforms of the leptin receptor exist. OB2Ra and OB2Rb are 2 major iso2forms of the OB2R. The extracellular and transmem2brae domains are identical between OB2Ra and OB2Rb, while the only difference is the length of the cy2toplasmic domain between them. The long isoforms have 302 amino acids cytoplamic residues and only 322 40 in the short isoforms. OB2Ra, the major OB2R short isoform, is expressed in many organs and is thought to play little role in signaling transduction but participates in leptin transport across the blood2brain barrier (BBB) and in leptin degradation. OB2Rb, re2ferred to as the long isoform of the OB2R, is primarily expressed in nuclei of the hypothalamus, a regulatory center for appetite control, and is considered to be a signaling2competent receptor isoform. OB2Rb has JAK and STAT proteins and works by activating the JAK/STAT pathway[6,7]. Recent reports have re2vealed that the expressions of OB2Rb mRNA can be

detected in various peripheral organs, which suggests that leptin has many peripheral actions, including suppression of insulin secretion, stimulation of cy2 tokine production and macrophage phagocytosis, and control of the development of reproductive sys2 tems[8,9]. This research demonstrated that 2 short isoforms of human leptin receptor, OB2R219. 1 and OB2R219. 3, were expressed in HepG2 cells, which suggests that the HepG2 cell line is a useful model for leptin receptors research[10].

The increased leptin level observed in obese chil2 dren supports the hypothesis that leptin resistance or insensitivity to leptin may be a common mechanism of human obesity[11]. One explanation for leptin resis2 tance is that the transport system that allows leptin to enter the brain is saturable. Studies on rodents have shown that the inhibition of food response to intrac2 erebroventricular injection of leptin was attenuated in rats with diet2induced obesity[12], which suggested that the responsiveness to leptin may vary according to metabolic conditions and leptin resistance could be relevant to leptin receptors or their downstream sig2 naling pathways. Recent studies have shown that lep2 tin induced a marked inhibition of OB2Rb expression in neuroblastoma cells[13], suggesting that leptin could down2regulate cerebral leptin receptors. The present study found that leptin (10^{-6} and 10^{-7} M) produced a significant inhibition on both OB2Ra and OB2Rb mRNA in HepG2 cells, which is partly con2 sistent with the reported study on chicken2derived leghorn mail hepatoma cells[14], implying that down2 regulation of leptin receptors induced by leptin may also occur in peripheral organs. Besides ligand2in2 duced receptor down2regulation, there may be other mechanisms underlying leptin2induced receptor down2 regulation, which need further study.

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