Leptin regulates proliferation and apoptosis of colorectal carcinoma through PI3K/Akt/mTOR signalling pathway

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Epidemiological studies have indicated that obesity is associated with colorectal cancer. The obesity hormone leptin is considered as a key mediator for cancer development and progression. The present study aims to investigate regulatory effects of leptin on colorectal carcinoma. The expression of leptin and its receptor Ob-R was examined by immunohistochemistry in 108 Chinese patients with colorectal carcinoma. The results showed that leptin/Ob-R expression was significantly associated with T stage, TNM stage, lymph node metastasis, distant metastasis, differentiation and expression of p-mTOR, p-70S6 kinase, and p-Akt. Furthermore, the effects of leptin on proliferation and apoptosis of HCT-116 colon carcinoma cells were determined. The results showed that leptin could stimulate the proliferation and inhibit the apoptosis of HCT-116 colon cells through the PI3K/Akt/mTOR pathway. Ly294002 (a PI3K inhibitor) and rapamycin (an mTOR inhibitor) could prevent the regulatory effects of leptin on the proliferation and apoptosis of HCT-116 cells via abrogating leptin-mediated PI3K/Akt/mTOR pathway. All these results indicated that leptin could regulate proliferation and apoptosis of colorectal carcinoma through the PI3K/Akt/mTOR signalling pathway.

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1. Introduction

Colorectal cancer is the third most common cancer in the world, and incidence rates are tenfold higher in developed than in developing countries (Key *et al.* 2004). In 2002, the International Agency for Research on Cancer (IARC) expert panel reported that there was a link between weight and cancer (Prieto-Hontoria *et al.* 2011). Epidemiological and cohort studies indicate that there is a strong correlation between obesity and risk of colorectal cancer (Siegel *et al.* 2010).

Leptin, a 167-amino-acid peptide mainly synthesized and released by adipocytes, has been demonstrated to play an important role in regulating food intake and energy expenditure (Ahima *et al.* 1996). Circulating serum leptin levels are elevated in people with high body mass index (BMI). Leptin has been shown to be able to regulate proliferation in various normal and neoplastic cell types (e.g. prostate, colon, ovarian and breast cancers, etc.) through activating mitogenic and anti-apoptotic signalling pathways (Onuma *et al.* 2003; Hoda and Popken 2008). Leptin exhibits its activity mainly through binding to its long form, fully active receptor (Ob-Rb), which can induce trans-autophosphorylation of cytoplasmic Janus kinase 2 (JAK2). Furthermore, activated JAK2 phosphorylates tyrosine residues on the cytoplasmic tail of Ob-R, which induces the recruitment of potential signal transducers and activators of transcription (STATs). Additionally, Ob-R has been reported to induce the activation of other signalling pathways, including Ras/Raf/mitogen-activated protein kinase

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(MAPK) and insulin receptor substrates (IRS1/2)/phosphoinositide 3-kinase (PIK3) pathways (Cirillo *et al.* 2008).

mTOR (mammalian target of rapamycin) is a core component of intracellular signalling for cellular growth, mRNA translation, and metabolism. mTOR has been demonstrated to be involved in gene regulation and controlling translation of specific transcripts when responding to environmental stimulin (Gingras *et al.* 2004). mTOR could be activated by PI3K/Akt pathways. Previous studies have showed that PI3K/mTOR signalling pathway plays a pivotal role in diabetes and inflammation (Maya-Monteiro and Bozza 2008; Maya-Monteiro *et al*. 2008).

The association between leptin's regulatory effect on colon cancer and PI3K/AKT/mTOR signal transduction pathway remains unclear. The aim of this study is firstly to investigate the correlation between the expression of leptin/Ob-R and clinicopathologic features in 108 patients with colorectal carcinoma. Furthermore, HCT-116 colon cancer cells were used to elucidate the effect of PI3K/Akt/mTOR signalling pathway on the leptin's regulation on colon cancer.

2. Materials and methods

2.1 *Reagents*

Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS) and penicilli-streptomycin were purchased from Gibco (Grand Island, NY). Recombinant human leptin was purchased from PeproTech, Inc (Rochy Hill, NY). Ly294002 and rapamycin were purchased from Sigma (St. Louis, MO, USA).

2.2 *Patients and samples*

108 patients with colorectal carcinoma diagnosed from 2002 to 2005 were selected from the First Hospital of Changsha. All the procedures were approved by the research ethics committee of the First Hospital of Changsha.

The patients included in this study were 57 males and 51 females. Their mean age was 58.9 years with a range from 19 to 86 years. The tumours were located in colon in 78 patients and rectum in 30 patients. The distribution by TNM stage at diagnosis was as followed: stage I in 24 patients, stage II in 27 patients, stage III in 45 patients, and stage IV in 12 patients.

2.3 *Immunohistochemical staining*

Sections (5 μm) were prepared from formalin-fixed, paraffin-embedded human specimen, deparaffinized and rehydrated. After blocking with 3% skim milk, the sections were incubated with primary antibodies which were listed as followed: H-146 rabbit polyclonal antibody (1:200 dilution) for leptin, B-3 mouse monoclonal antibody (1:250 dilution) for ObR, D9E rabbit polyclonal antibody (1:100 dilution) for phosphor-Akt (Ser 473), rabbit polyclonal antibody (1:80 dilution) for phosphor-GSK3 (Ser 21/9), rabbit polyclonal antibody (1:100 dilution) for phosphor-70S6 kinase (Thr 389), rabbit polyclonal antibody (1:250 dilution) for phosphor-mTOR (Ser 2448). Sections were then washed and incubated with biotinylated secondary antibody for 30 min.

The immunohistochemical (IHC) staining was evaluated and photographed using an Olympus CX42 microscope. Two independent investigators scored the expression of leptin and ObR. A scale of 0, + (weak inmmunoreactivity), ++(moderate immunoreactivity) and +++ (robust immunoreactivity) was applied according to the intensity of immunolabelling and number of positive cells. Tissues scoring at least 1 plus (+) was considered to be positive, while that below 1+ was considered to be negative. In the above IHC analysis, there was no discrepancy between the two observers regarding the patterns of biomarker expression and the scores assigned to analysed sections.

p-Akt, p-GSK3, p-mTOR, p-70S6K protein expression in those 108 cases was evaluated by two pathologists with prior knowledge of the patients' clinical data using Olympus CX42 microscope (Olympus Optical). Specifically, underexpression was defined as no staining or staining intensity in tumour tissue being less than matched normal tissue, a normal expression as staining intensity being similar to matched normal tissue and overexpression as staining intensity being higher than matched normal tissue.

2.4 *Cell culture and viability assays*

HCT-116, an undifferentiated and very invasive cell line derived from colon carcinoma, was obtained from Cancer Research Institute of Central South University. Cells were cultured at 37°C in a humidified atmosphere of 5% CO_2/air in DMEM supplemented with 10% FBS. Cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay according to the manufacturer's protocol. All experiments were carried out in triplicates.

2.5 *Treatment*

For all experimental procedures, cells were transferred to serum-free medium or 10% serum-containing medium 24 h after seeding. After a further 24 h, various concentrations of leptin were added for different treatment times. When inhibitors were employed, cells were pretreated for 3 h with inhibitor (LY294002, 20 μM; Rapamycin, 20 μM) before the addition of leptin.

2.6 *Colony formation assay*

Crystal violet (CV) staining of cells and clone counting were used to measure cell proliferation. HCT-116 cells were seeded in the 6-well plates with 2 mL medium (1000 cells/well). 200 ng/mL of leptin was added to HCT-116 cells. After 3 weeks, colonies were fixed with 4% formaldehyde and stained with 0.05% CV. The plates were carefully rinsed using ddH_2O until no colour appeared. Colony forming efficiency (CFE) was calculated by the percentage of colonies per well.

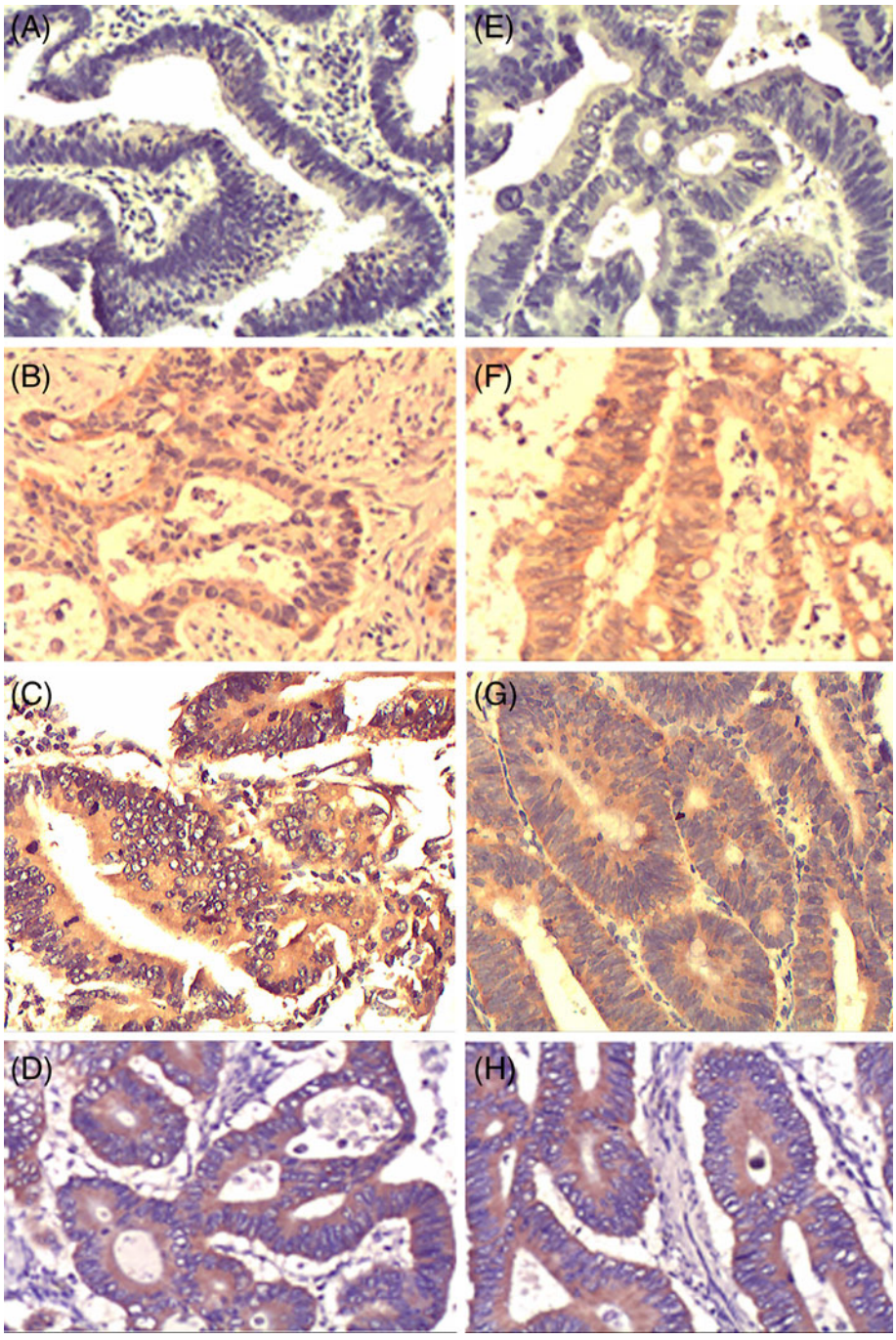

Figure 1. Representative figures of expression of leptin and Ob-R. (**A**) The expression of leptin at 0 level. (**B**) The expression of leptin at+level. (**C**) The expression of leptin at ++ level. (**D**) The expression of leptin at +++ level. (**E**) The expression of Ob-R at 0 level. (**F**) The expression of Ob-R at+level. (**G**) The expression of Ob-R at ++ level. (**H**) The expression of Ob-R at +++ level. The magnification is 200-fold.

Table 1. Associations between the expression of leptin and clinicopathologic factors in colorectal cancer

Factors	No. of cases	Leptin positive *n* (%)	Leptin negative *n* (%)	*P*-value
Gender				
Male	57	38 (66.7)	19 (33.3)	0.261
Female	51	39 (76.5)	12 (23.5)	
Age (years)				
<60	41	28 (68.3)	13 (31.7)	0.589
>=60	67	49 (73.1)	18 (26.9)	
T stage				
T1/T2	26	12 (46.2)	14 (53.8)	0.001
T3/T4	82	65 (79.3)	17 (20.7)	
TNM stage				
I/II	51	27 (52.9)	24 (47.1)	<0.001
III/IV	57	50 (87.7)	7 (12.3)	
Lymph node metastasis				
pN(−)	58	33 (56.9)	25 (43.1)	<0.001
pN(+)	50	44 (88.0)	6 (12.0)	
Distant metastasis				
Negative	97	66 (68.8)	31 (31.2)	0.026
Positive	11	11 (100)	0 (0.0)	
Differentiation				
Well	10	4 (40.0)	6 (60.0)	<0.001
Moderate	56	33 (58.9)	23 (41.1)	
Poor	42	40 (95.2)	2 (4.8)	
p-mTOR				
Negative	42	17 (40.5)	25 (29.5)	<0.001
Positive	66	60 (90.9)	6 (9.1)	
p-70S6 kinase				
Negative	46	7 (15.2)	39 (84.8)	<0.001
Positive	62	50 (80.6)	12 (19.4)	
p-Akt				
Negative	30	12 (40.0)	18 (60.0)	<0.001
Positive	78	65 (83.3)	13 (16.7)	
p-GSK3B				
Negative	38	17 (44.7)	21 (55.3)	<0.001
Positive	70	60 (85.7)	10 (14.3)	
LRb				
Negative	51	27 (52.9)	24 (47.1)	<0.001
Positive	57	50 (87.7)	7 (12.3)	

The *P*-values represent the comparison of leptin expression between different groups.

2.7 *Annexin V-FITC/PI (propidium iodide) analysis*

Flow cytometric analysis was performed to identify and quantify the apoptotic cells by using Annexin V-FITC/PI (propidium iodide) method. In brief, after HCT-116 cells were treated with the various concentrations of reagents, both adherent and floating cells were harvested and stained with Annexin V-FITC/PI according to the manufacturer's procedure. The samples were analysed with a Becton Dickinson FACSVantage SE instrument.

2.8 *Soft agar colony assay*

Soft agar colony formation assay was used to assess the anchorage-independent growth ability of cells. Cells were suspended in 1 mL of medium containing 0.3% low-melting-temperature agarose and 10% FBS. Cells were overlaid onto a bottom layer of solidified 0.6% agarose in medium in the absence or presence of leptin (200 ng/mL), at a concentration of 1000 cells per well. Colonies>50 μm were counted 14 days after plating. The experiments were performed in triplicates.

Table 2. Associations between the expression of LRb and clinicopathologic factors in colorectal cancer

Factors	No. of cases	LRb positive *n* (%)	LRb negative *n* (%)	*P*-value
Gender				
Male	57	30 (52.6)	27 (47.4)	0.974
Female	51	27 (52.9)	24 (47.1)	
Age (years)				
<60	41	20 (48.8)	21 (51.2)	0.515
>=60	67	37 (55.2)	30 (44.8)	
T stage				
T1/T2	26	8 (30.8)	18 (69.2)	0.01
T3/T4	82	49 (59.8)	33 (40.2)	
TNM stage				
I/II	51	18 (35.3)	33 (64.7)	0.001
III/IV	57	39 (68.4)	18 (31.6)	
Lymph node metastasis				
pN(−)	58	21 (36.2)	37 (63.8)	<0.001
pN(+)	50	36 (72.0)	14 (28.0)	
Distant metastasis				
Negative	97	47(48.5)	50 (51.5)	0.008
Positive	11	10 (90.9)	1 (9.1)	
Differentiation				
Well	10	2(20.0)	8 (80.0)	<0.001
Moderate	56	18 (32.1)	38 (67.9)	
Poor	42	37 (88.1)	5 (11.9)	
p-mTOR				
Negative	42	15 (35.7)	27 (64.3)	0.005
Positive	66	42 (63.6)	24 (36.4)	
p-70S6 kinase				
Negative	46	7 (15.2)	39 (84.8)	<0.001
Positive	62	50 (80.6)	12 (19.4)	
p-Akt				
Negative	30	10 (30.3)	20 (66.7)	0.012
Positive	78	47 (60.2)	31 (39.8)	
pGSK3B				
Negative	38	6 (15.8)	32 (84.2)	<0.001
Positive	70	51 (72.9)	19 (17.1)	
Leptin				
Negative	31	7 (22.6)	24 (77.4)	<0.001
Positive	77	50 (64.9)	27 (35.1)	

The *P*-values represent the comparison of leptin expression between different groups.

2.9 *Reverse transcription polymerase chain reaction (RT-PCR)*

Total RNA was extracted using TRIzol reagent (invitrogen, USA). The RNA yield and purity were assessed by spectrophotometric analysis. Total RNA (3 μg) from each sample was subjected to reverse transcription using the One-Step RT-PCR System (Fermentas, Vilnius, Lithuania). The PCR of cDNA was performed using Takara Ex Taq Hotstart polymerase, dNTPs and the following related primers: 5′-CCATGATCATTTTATCCCCAT-3′ (sence) and 5′-TTGGCACATTGGGTTCATCT-3′ (antisense) for Ob-Rt. 5′-TGGTGAAGAACAAGGGCTTA-3′ (sense) and 5′- AATAAACAGGGGGCTGGGAAT-3′ (antisense) for Ob-Rb. 5′-AATCCCATC ACCATCTTCCA-3′ (sense) and 5′- CCTGCTTCACCACCTTCTTG-3′ (antisense) for GAPDH. After denaturation for 1 min at 95°C, the total amount of products was amplified for 30 cycles for LRa and LRb, and 25 cycles for GAPDH (95°C, 30 s; 60°C, 30 s; 68°C, 90 s).

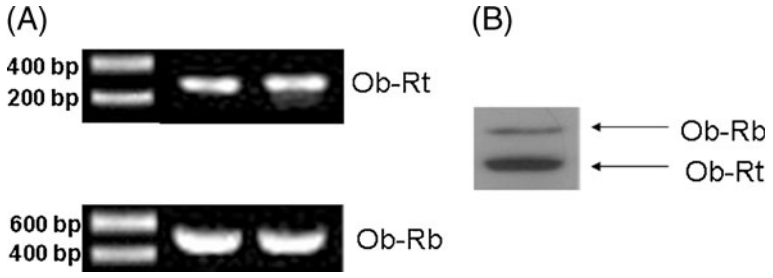

Figure 2. Identification of the expression of Ob-Rb (long form of Ob-R) and Ob-Rt (short form of Ob-R). (**A**) Detection of mRNA using RT-PCR. (**B**) Western blotting analysis of protein expression.

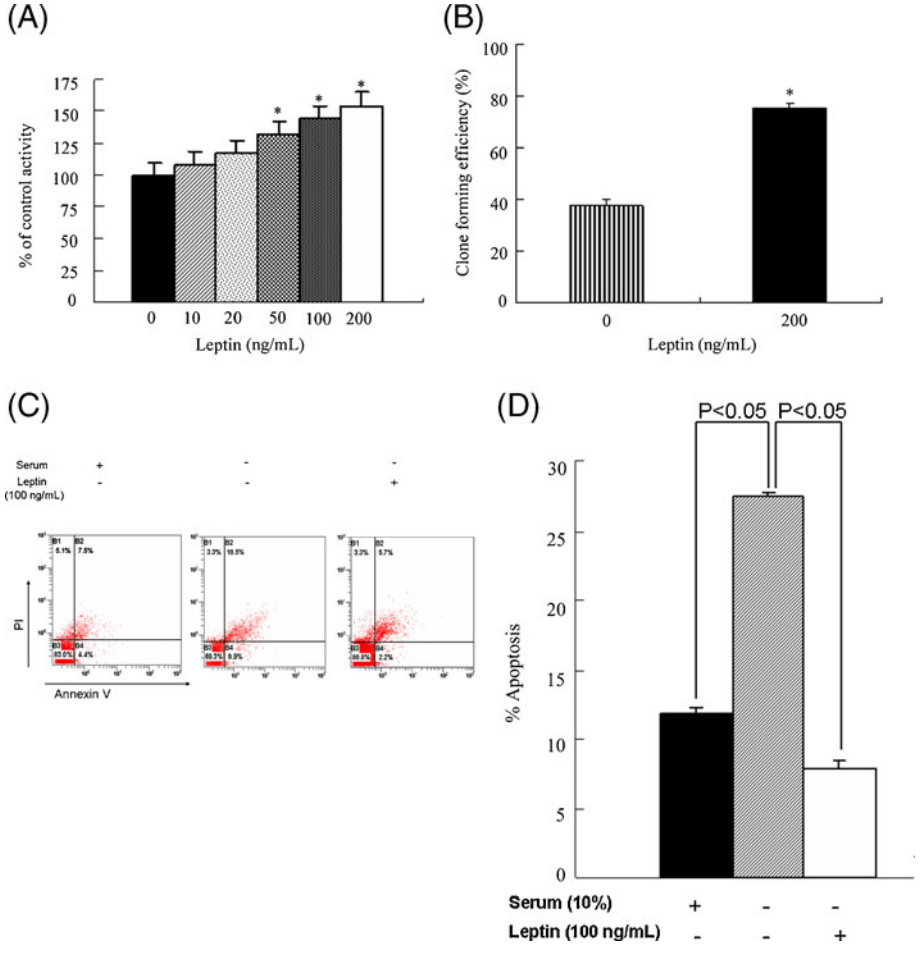

Figure 3. Effects of leptin on proliferation and apoptosis of HCT-116 cells. (**A**) Leptin increases cell proliferation in HCT-116 cells in a dose-dependent manner. After 48 h, cell proliferation was determined using MTT assay as described in the materials and methods section. Data were given as mean±S.D. (n=3). *P<0.05, compared with control. (**B**) The effect of leptin (200 ng/mL) on cell colony formation. Data were given as mean±S.D. (n=3); *P<0.05, compared with control. (**C**) Representative figure of flow cytometric analysis of Annexin V/PI double-stained cells. In each plot, the lower right quadrant represents early apoptotic cells, and the upper right quadrant represents late apoptotic cells. (**D**) Quantitative evaluation of apoptosis was carried out by flow cytometry with Annexin V/PI double-staining, and shown as the percentage of Annexin V positive cells. Results are the mean±S.D. (n=3).

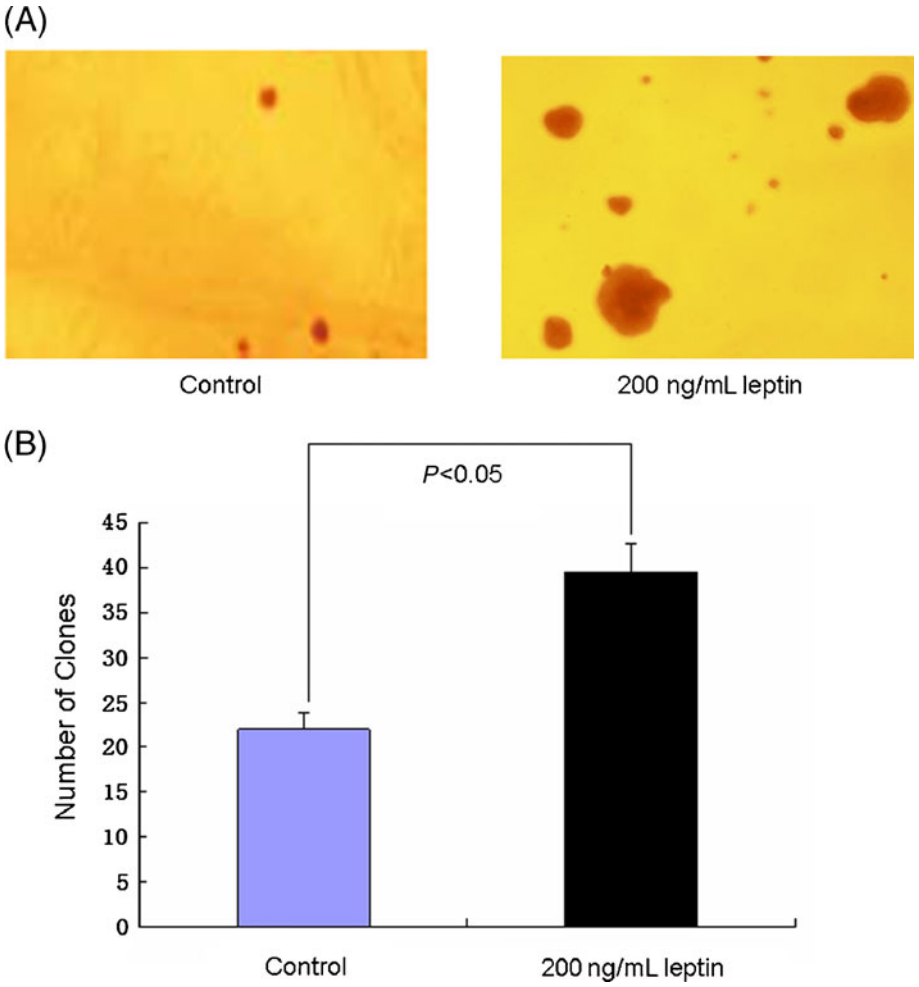

Figure 4. The effect of leptin (200 ng/mL) on anchorage-independent cell growth of HCT-116. (**A**) A representative photograph of soft agar plates at 14 days. Addition of leptin (200 ng/mL) resulted in the elevation of colony formation. (**B**) Quantitative assessment of soft agar colony growth.

2.10 *Western blot analysis*

Whole cell lysates were extracted from cultured HCT-116 cells, and protein concentrations were determined using Bio-Rad Protein Assay (Bio-Rad, Hercules, CA). Protein samples (50–150 μg) were separated on SDS-polyacrylamide gel and transferred to nitrocellulose membranes. The membranes were incubated with polyclonal antibodies for Akt (1:1000), phosphorylated Akt (Phospho-Akt Ser473, 1:1000), p70S6 kinase (1:1000), phosphorylated p70S6 kinase (Ser389, 1:500), for phospho-NF-kB, NF-KB P65 pAb, 1:1000, for total NF-κB P65 pAb, 1:1000. For ObR, we used B-3 mouse mAb, 1:200 (Santa Cruz); and for mTOR pAb;1:1000 (P2476, Bioworld Technology, Inc, USA) and p-mTOR pAb, 1:1000 (ser-2448; Bioworld Technology, Inc, USA), anti-β-actin antibody was used as a loading control.

2.11 *Statistical analysis*

All statistical analyses were done using the SPSS 10.0 software package (SPSS Inc., Chicago, IL, USA). Differences between groups were compared using χ^2 tests. All tests were two-tailed and were considered significant at $P<0.05$.

3. Results

3.1 *Association between leptin expression and clinicopathologic characteristics of colorectal cancer*

The representative figure of leptin expression is given in figure 1A–D. The association between leptin expression and clinicopathologic characteristics of colorectal cancer is given in table 1. Leptin expression is significantly associated

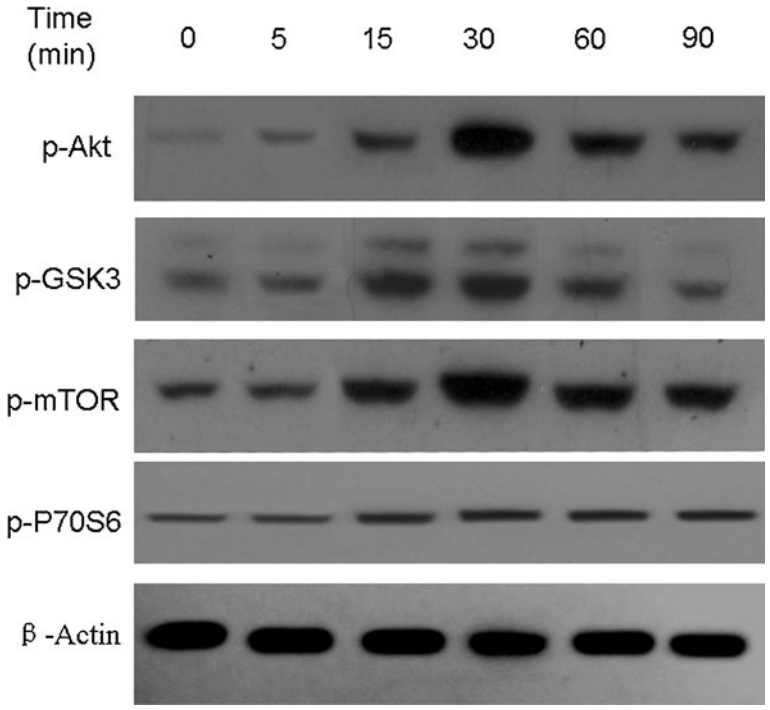

Figure 5. The influence of leptin on phosphorylation of Akt, GSK3, mTOR and P70S6K. HCT-116 cells were left treated with leptin (100 ng/mL) for 0, 5, 15, 30, 60 and 90 min. The phosphorylation of these proteins was evaluated by western blotting.

with T stage (P=0.001), TNM stage (P<0.001), lymph node metastasis (P <0.001), distant metastasis (P=0.026), differentiation (P<0.001), and expression of p-mTOR (P<0.001), p-70S6 kinase (P<0.001), p-Akt (P<0.001), p-GSK3β (P<0.001), and Ob-R (P<0.001). However, leptin expression is not associated with the age (P=0.589) and gender (P=0.261) of patients.

3.2 *Association between Ob-R expression and clinicopathologic characteristics of colorectal cancer*

The representative figure of leptin expression is given in figure 1E–H. The association between Ob-R expression and clinicopathologic characteristics of colorectal cancer is given in table 2. Ob-R expression is significantly associated with T stage (P=0.01), TNM stage (P=0.001), lymph node metastasis (P<0.001), distant metastasis (P=0.008), differentiation (P<0.001), and expression of p-mTOR (P=0.005), p-70S6 kinase (P<0.001), p-Akt (P=0.012), p-GSK3β (P<0.001), and leptin (P<0.001). However, Ob-R expression is not associated with the age (P=0.515) and gender (P=0.974) of patients.

3.3 *Leptin stimulates proliferation and reduces apoptosis of HCT-116*

As shown in figure 2, the long and short isoforms of Ob-R are expressed in HCT-116 cells.

The effect of leptin on the proliferation and apoptosis of HCT-116 was firstly investigated. HCT-116 cells were serum-starved and subsequently treated with leptin at increasing concentrations (10–200 ng/mL) for 48 h. As shown in figure 3A, compared with control, the cell viability significantly increased when HCT-116 cells were treated with 50, 100 and 200 ng/mL of leptin for 48 h (P<0.05). Further colony formation assay (figure 3B) showed 200 ng/mL of leptin significantly elevated the cell colony formation (P<0.05).

To evaluate the influence of leptin on the apoptosis of HCT-116 cells, Annexin V-FITC/PI (propidium iodide) staining analysis was employed. In the dual parameter fluorescent dot plots, the cells in early apoptosis (annexin V^+/PI^-, in the lower right quadrant) and in the late apoptosis (annexin V^+/PI^+, in the upper right quadrant) were counted. As shown in figure 3C and D, serum starvation significantly increased the apoptosis of HCT-116 cells. However, treatment of HCT-116 cells with leptin (100 ng/mL) significantly prevented serum-starved apoptosis (P<0.05).

3.4 *Leptin increases anchorage-independent cell growth*

Anchorage-independent cell growth in soft agar represents tumorigenicity of cells *in vitro* and correlates with the malignant potential of cells *in vivo*. As shown in figure 4A and B, addition of leptin (200 ng/mL) significantly increased the number of colonies counted.

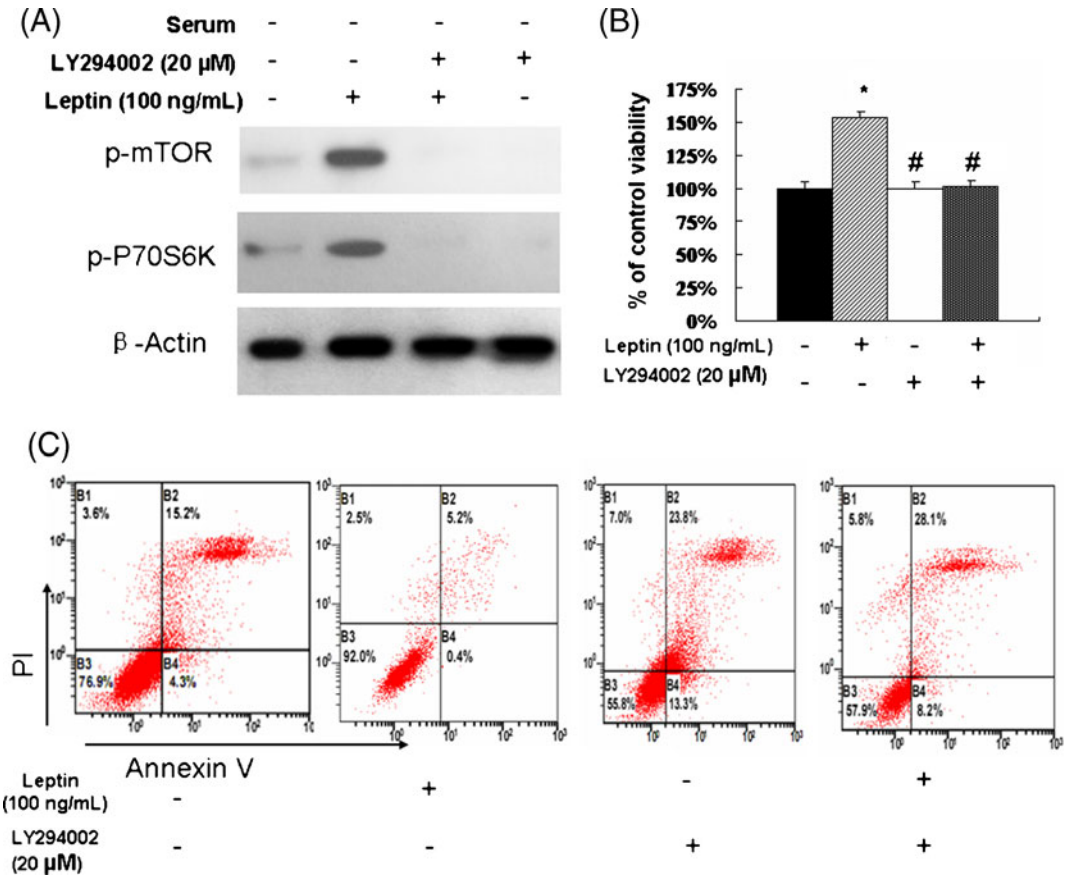

Figure 6. Inhibitor of PI3K (Ly294002) attenuates leptin-induced cell proliferation, and abolishes the antiapoptotic action of leptin. HCT-116 cells were cultured in serum-free medium for 24 h with or without leptin (100 ng/mL). Before adding leptin, cells were pretreated with Ly294002 (20 μM). (**A**) The effect of Ly294002 (20 μM) on the leptin-induced phosphorylation of mTOR and P70S6K. (**B**) Ly294002 (20 μM) attenuates leptin-induced cell proliferation. *$P<0.05$ compared with control; #$P<0.05$ compared with leptin-treated group. (**C**) Ly294002 (20 μM) abolishes leptin-induced antiapoptotic action. Representative figure of flow cytometric analysis of Annexin V/PI double stained cells was given.

3.5 *Leptin activates the PI3K/AKT/ mTOR pathways in HCT-116 colon cancer cells*

As shown in figure 5, in HCT-116 cancer cells, leptin (100 ng/mL) stimulated the phosphorylation of Akt, GSK3, mTOR and P70S6 kinase at different treatment time. After treatment with leptin (100 ng/mL) for 30 min, the levels of p-Akt, GSK3, mTOR and P70S6 reached the maximum.

3.6 *The influence of mTOR and PI3K inhibitor on leptin-induced proliferation and decreased apoptosis of HCT-116 cells*

To further clarify the importance of PI3K/AKT/mTOR signalling pathway in leptin-induced proliferation and decreased apoptosis of HCT-116 cells, we examined the ability of the PI3K inhibitor LY294002 and the mTOR inhibitor rapamycin to inhibit the regulatory effect of leptin. As shown in figure 6A, LY294002 (20 μM) prevented the elevation of p-mTOR and p-P70S6 kinase levels induced by leptin (100 ng/mL). Compared with leptin-treated group, treatment of with LY294002 (20 μM) significantly increased the apoptosis and reduced the proliferation of HCT-116 cells (figure 6B and C). Similar results were also detected in mTOR inhibitor rapamycin (figure 7). Rapamycin (20 μM) prevented the elevation of p-mTOR and p-P70S6 kinase levels induced by leptin (100 ng/mL) (figure 7A). Compared with the leptin-treated group, treatment of with rapamycin (20 μM) significantly increased the apoptosis and reduced the proliferation of HCT-116 cells (figure 7B and C).

4. Discussion

The prevalence of obesity is increasing worldwide, which has prompted researchers to investigate the relationship

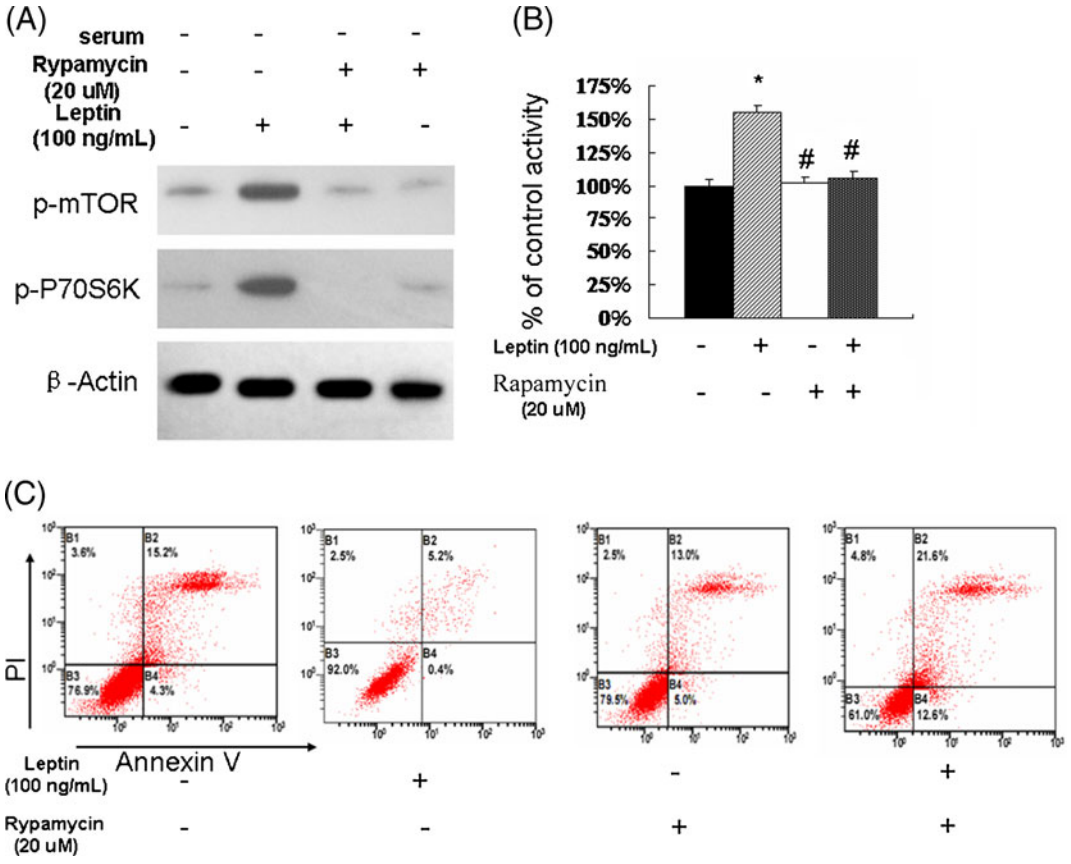

Figure 7. Inhibitor of mTOR (Rypamycin) attenuates leptin-induced cell proliferation, and abolishes the antiapoptotic action of leptin. HCT-116 cells were cultured in serum-free medium for 24 h with or without leptin (100 ng/mL). Before adding leptin, cells were pretreated with Rypamycin (20 μM). (**A**) The effect of Rypamycin (20 μM) on the leptin-induced phosphorylation of mTOR and P70S6K. (**B**) Rypamycin (20 μM) attenuates leptin-induced cell proliferation. *$P<0.05$ compared with control; #$P<0.05$ compared with leptin-treated group. (**C**) Rypamycin (20 μM) abolishes leptin-induced antiapoptotic action. Representative figure of flow cytometric analysis of Annexin V/PI double-stained cells was given.

between obesity and various diseases and the detailed mechanisms involved in their interaction. The link between leptin and cancer has been demonstrated in various cancers (Ishikawa *et al.* 2004; Garofalo *et al.* 2006; Zhao *et al.* 2007; Uddin *et al.* 2009), and leptin receptors have also been detected on various cancer cells (Ishikawa *et al.* 2004; Choi *et al.* 2005).

In this study, the expression of leptin and its receptor Ob-R was firstly determined in 108 Chinese patients with colorectal carcinoma and associated with clinicopathologic factors. Our data showed that patients with high leptin/Ob-R expression showed later-stage, easy metastasis and poor differentiation, which indicated that there is a strong relationship between leptin/Ob-R expression and colon cancer malignancy. Further correlation analysis showed that leptin/Ob-R expression was strongly associated with signalling molecules in PI3K/Akt/mTOR pathway, including p-mTOR, p-70S6 kinase, p-Akt and p-GSK3B. That indicates that leptin/Ob-R expression was linked to PI3K/Akt/mTOR signalling pathway in colorectal carcinoma.

Furthermore, HCT-116 colon cell lines were employed to study the relationship between leptin and PI3K/Akt/mTOR signalling pathway. The oncogenic effect of leptin on HCT-116 cells was clearly demonstrated, and increased cell proliferation and inhibition of apoptosis induced by leptin might be the major reasons. Previous studies have reported that leptin possesses proliferation-inducing and anti-apoptotic activity (Liu *et al.* 2001; Hardwick *et al.* 2001; Chen *et al.* 2011), which strongly supports our recent findings. Additionally, we also observed that leptin could increase anchorage-independent cell growth of HCT-116. Furthermore, the influence of leptin on signalling molecules in PI3K/Akt/mTOR pathway was determined. Our experimental data showed that leptin induced the phosphorylation of Akt, GSK3, mTOR and P70S6 kinase, which indicated that this key signal transduction pathway was activated by

leptin. Additionally, the PI3K inhibitor (LY294002) and the mTOR inhibitor (rapamycin) could prevent leptin-induced activation of PI3K/Akt/mTOR signalling pathway, which resulted in prevention of leptin-induced cell proliferation and decreased apoptosis of HCT-116 cells.

In conclusion, our recent results demonstrated that leptin could regulate proliferation and apoptosis of colorectal carcinoma through PI3K/Akt/mTOR signalling pathway. Given the fact that leptin signalling pathway could also be transduced by other signalling pathways including JAK/STAT and MAPK pathways, further experiments should be carried out to investigate the relationship between leptin regualtion of colorectal carcinoma and other signalling pathways.

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