Leptin and its receptor in glucose metabolism of T-cell lymphoma

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Abstract. T-cell lymphoma (TCL) is a group of heterogeneous disorders with a poor response to conventional treatment. In order to identify novel therapeutic targets, the present study investigated the effect of leptin and its receptor on glucose metabolism in TCL. The expression of the leptin receptor (ObR), and glucose transporter (Glut)1 and 4 was detected in TCL and reactive lymphoid hyperplasia (RLH) tissues by immunohistochemical analysis. A higher level of ObR expression was observed in the TCL tissues than in the RLH tissues (58.3 vs. 22.2%; P=0.012), and ObR overexpression was associated with high expression of Glut1 (P=0.007). *In vitro* analysis using the human TCL MOLT-3 cell line demonstrated that leptin stimulated cell glucose uptake via promoting recruitment and expression of Glut1, effects which were abolished by ObR-specific small interfering RNA (siRNA). Additionally, MOLT-3 cell viability was also increased following leptin treatment. ObR-specific siRNA abolished these responses. In conclusion, these results suggested that leptin serves a critical role in TCL glucose uptake via the ObR.

Introduction

T-cell lymphoma (TCL) originates from T-cells or natural killer (NK) cells at various developmental stages, accounting for 10-15% of cases of non-Hodgkin's lymphoma (1). In China, mature T/NK-cell neoplasms account for 23.3% of lymphoid neoplasms (2), a relatively higher proportion than in Western countries (3). TCL consists of a group of diseases with high heterogeneity, with more than twenty subtypes of T-cell neoplasms with a particularly aggressive course, based on the World Health Organization (WHO) classification (4). Compared with malignancies derived from B cells, TCL is more resistant to conventional treatments, which are based on the mechanism of interfering with the cell cycle and proliferation, including CHOP and ESHAP (5), leading to the unfavorable prognoses of patients. Therefore, it is necessary to investigate the biochemical metabolism characteristics and mechanisms of malignant T cells, in order to identify novel therapeutic targets for patients with aggressive disease.

Glucose is the major energy source used to maintain cell proliferation and homeostasis. In cancer, glucose metabolism serves an even more important role than in normal tissue, due to the faster cell proliferation and greater calorific demand of malignant cells (6). Glucose metabolism is not only involved in the proliferation and apoptosis of tumor cells, but also affects tumor response to chemotherapy. Genetic deletion of glucose transporters (Gluts) in B-cell acute lymphoblastic leukemia cells leads to reduced glucose uptake, decreased cell proliferation and increased apoptosis (7). In T-cell acute lymphoblastic leukemia, 2-deoxyglucose, an inhibitor of glycolysis accelerates glucocorticoid-induced tumor cell death, while a high concentration of glucose suppresses the etoposide chemotherapy effect on B-cell lymphoma through BCL-6 (8).

A number of cytokines and adipokines participate in the glucose metabolism of tumor malignant cells. Leptin, the product of the obese (ob) gene, is the most widely studied adipokine (9). This secreted protein, composed of 146 amino acids, exerts actions through its specific receptor (leptin receptor, ObR), which is localized to the cell membrane and is present in a variety of hematopoietic cells, including hematopoietic progenitor cells, erythropoietic, myeloid and lymphoblastic cells (10). Leptin affects glucose metabolism at the central and peripheral levels in healthy bodies. In the central nervous system, leptin regulates glucose metabolism primarily through hypothalamic neurons, including glucose-excited neurons and glucose-inhibited neurons (11). At the peripheral level, leptin directly affects β cells of pancreas and affects insulin secretion (12).

A recent study has investigated the effects of leptin on glucose metabolism in normal T cells and have achieved inconsistent results; however, the role of leptin in malignant T cells remains unreported (13,14). As glucose metabolism is involved in the proliferation and apoptosis of malignant cells and may affect the prognosis of the malignancies, we hypothesized that leptin may promote the glucose uptake of TCL cells. Therefore, the present study was performed in order to verify this hypothesis, with the expectation of identifying novel treatment strategies to improve the prognosis of aggressive TCL. In

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order to investigate the effect of leptin on the glucose metabolism of TCL cells, the present study analyzed the expression of ObR and Gluts in TCL cells lines and tissues, and observed the changes in the proliferation and glucose consumption of TCL MOLT-3 cells following recombination human leptin (rhleptin) intervention. The decreased expression of ObR was further detected by small interfering RNA (siRNA) and the subsequent effect on glucose consumption *in vitro*.

Materials and methods

Patient samples. Tissues from 36 patients with de novo diagnosed TCL (mean age 58.5 years, range 21-82 years; male/female ratio was 23/13) and 18 with RLH were obtained between January 2008 and January 2013 from the Department of Pathology at Tai'an City Central Hospital (Tai'an, China). All cases were reclassified according to WHO criteria (4). The present study was conducted with the approval of the the ethical committee of Tai'an central hospital (Tai'an, China) and written informed consent was obtained from all participants, including the patient from whom peripheral blood mononuclear cells (PBMCs) were obtained.

Immunohistochemistry. The 10% formalin-fixed (at room temperature for 6 h) paraffin-embedded tissue samples were sliced into 3-μm sections which were deparaffinized and subsequently rehydrated in a descending alcohol series. Antigens were heat-retrieved at 98˚C in EDTA solution. Following cooling to room temperature, the tissue sections were quenched with 3% hydrogen peroxidase and non-specific binding sites were blocked with 5% goat serum at 37˚C for 30 min. Subsequently, the sections were incubated with the following primary antibodies: ObR (dilution, 1:100 cat. no., ab2139; Abcam, Cambridge, UK), Glut1 (dilution, 1:100; cat. no. ab115730, Abcam) and Glut4 (dilution, 1:50 cat. no. BA1626; Boster Biological Technology., Co., Ltd., Beijing, China) at 4˚C overnight. Following washing in phosphate-buffered saline, slides were incubated with a secondary antibody (SPlink Detection kits SP 9001 and SP 9002; ZSJQ-BIO, Co., Ltd, Beijing, China) at room temperature for 1 h, prior to being incubated with ABC reagent (SPlink Detection kits SP 9001 and SP 9002), according to the manufacturer's protocol. The peroxidase activity was visualized using a Histofine 3,3'-diaminobenzidine substrate kit (OriGene Technologies, Inc., Beijing, China). The sections were counterstained at room temperature for 3 min with hematoxylin, prior to being dehydrated and mounted on slides. Immunohistochemistry assessment based on the staining intensity and the proportion of positive tumor cells, ObR, Glut1 and Glut4 expression levels were assessed by two expert pathologists who were blinded to the clinical data. The staining degree is evaluated according to the extent and intensity of the staining under a laser scanning confocal microscope (magnification, x400). Cell staining was scored as follows: No cell staining, 0; <25% stained cells, 1; 25-50% stained cells, 2; and >50% stained cells, 3. Intensity of staining was scored as follows: Uncolored, 0; light brown, 1; brown, 2; and dark brown, 3. The results of the two aforementioned scoring systems were subsequently combined: ≤1 was defined as negative staining, 2-3 was defined as weak positive staining and ≥4 was defined as positive staining.

Cell culture. The human TCL Jurkat, HUT-78 and MOLT-3 cell lines were cultured in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA), supplemented with 10% fetal bovine serum (FBS; Hyclone; GE Healthcare Life Sciences, Logan, UT, USA). All cell lines were provided by Central Laboratory of Shangdong Provincial Hospital. Cells were maintained in a humidified atmosphere in 5% CO_2 at 37˚C, and the culture medium was changed every 2-3 days. For leptin and low/high concentration of glucose, cells were serum-starved for 24 h and were then treated with leptin at 0, 10, 100 and 200 ng/ml, in triplicate at 37˚C for 24, 48, 72 and 96 h.

Extraction of PBMCs. The PBMCs of a 32-year old healthy male donor were extracted in our laboratory in October 2013. Fresh blood was mixed with sterilized normal saline and lymphocyte separation medium (cat. no. P8610, Solarbio Science and Technology Co., Ltd., Beijing, China) (1:1:1). The mixture was centrifuged at 670 x g for 20 min at room temperature. The film layer was resuspended in saline (1:3), and centrifuged at 377 x g for 10 min at room temperature. The supernatant was discarded, another 10 ml saline was added and the mixture was centrifuged again at 377 x g for 10 min at room temperature. PBMCs were found in the resulting pellet.

Reagents and antibodies. Recombinant human leptin was purchased from PeproTech EC Ltd. (London, UK) and dissolved in sterile water, according to the manufacturer's protocols. NanoFectin™ was purchased from Shanghai ExCell Biology, Inc. (Shanghai, China). Mouse anti-ObR and rabbit anti-Glut1 monoclonal antibodies were purchased from Abcam (Cambridge, UK). Mouse anti-Glut4 polyclonal antibodies were purchased from BIOSS (Beijing China). The mouse anti-β-actin polyclonal antibody was purchased from OriGene Technologies, Inc.

Cell proliferation assay. Cell proliferation was measured by the Cell Counting kit-8 (CCK-8) assay (EnoGene Biotech Co., Ltd., Nanjing, China). To determine the effect of glucose concentration on cell proliferation, MOLT-3 cells were seeded onto a 96-well plate ($5x10^4$ cells/100 μl/well), using RPMI-1640 medium with a low (1,000 mg/l) or high (4,500 mg/l) glucose concentration. Next, cells at the same density were exposed to 0, 10, 100 and 200 ng/ml rhleptin at 37˚C for 24, 48 and 72 h in a humidified atmosphere in a 5% CO_2 incubator. Replicates of 6 wells for each dosage were analyzed for each experiment. The cells were subsequently incubated with 10 μl CCK-8 for 4 h at 37˚C. The optical density (OD) was subsequently measured at 450 nm on a scanning multi-well spectrophotometer. The cell viability rate was calculated according to the following equation: Cell viability rate (%)=(OD experiment-OD blank)/(OD control-OD blank) x100.

Glucose uptake assay. MOLT-3 cells ($2x10^5$ cells/1,000 μl/well) were cultured with, 10, 100 and 200 ng/ml rhleptin at 37˚C in a 24-well plate. Following treatment, the cell suspension in each well was collected and centrifuged (8,000 x g for 5 min at room temperature). The supernatant was then detected using a Glucose (HK) assay kit (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany), according to the manufacturer's protocols. The detection was performed using a spectrophotometer at 340 nm. The values were calculated according to the standard curve and were then subtracted from the base line to obtain values of glucose consumption from the total amount of glucose in the medium (without cells).

Assay for membrane protein level of ObR, Glut1 and Glut4. MOLT-3 cells were cultured without serum in a 24-well plate overnight and were subsequently incubated for increasing time periods (0, 15, 30, 45 or 60 min) with 100 ng/ml leptin at 37˚C. Next, cells were collected and cell membrane proteins were extracted using a Membrane Protein Extraction kit (Beyotime Institute of Biotechnology, Haimen, China). The membrane protein expression levels of ObR, Glut1 and Glut4 were detected though western blot analysis.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted from Molt-3 cells after culture for 48 h using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc., Waltham MA, USA). Next, cDNA was synthesized with 1 µg total RNA using the Takara RT reagents (Takara Biotechnology Co., Ltd., Dalian, China). Reverse transcription reactions were conducted at 37˚C for 15 min, then 85˚C for 5 sec. Primers were obtained from Takara Biotechnology Co., Ltd., the sequences of which were as follows: ObR forward, 3'-CATTTTATCCCCATTGAG AAGTA-5' and reverse, 3'-CTGAAAATTAAGTCCTTGTGC CCAG-5'; Glut1 forward, 3'-CTTTGTGGCCTTCTTTGA AGT-5' and reverse, 3'-CCACACAGTTGCTCCACAT-5'; Glut4 forward, 3'-CTTCCAACAGATAGGCTCCG-5' and reverse, 3'-CCCCAATGTTGTACCCAAAC-5'; and β-actin forward, 3'-TGACGTGGACATCCGCAAAG-5' and reverse, 3'-CTGGAAGGTGGACAGCGAGG-5'. Amplification reactions were performed using SYBR Premix Ex Taq (Takara Biotechnology Co., Ltd.) using the Roche LightCycler 480 qPCR system. Expression data were normalized to the geometric mean of the housekeeping gene β-actin to control the variability in expression levels. For data analysis, the $2^{-\Delta\Delta Cq}$ method was used. qPCR for each gene of each cDNA sample was assayed in triplicate. ΔCq=Cq (target gene)-Cq (β-actin gene); ΔΔCq=ΔCq (As2S2-treated cells)-ΔCq (untreated control) (15).

Western blot analysis. Membrane proteins were extracted by Membrane Protein Extraction kit (cat. no., BB-3116-2, Bestbio, Co., Ltd, Shanghai, China). Total protein was extracted from TCL cells using radioimmunoprecipitation assay buffer and 1% PMSF (both Shanghai Shenergy Biocolor Science & Technology Company, Shanghai, China). The protein concentration of the samples was determined using a bicinchoninic acid assay kit (Shanghai Shenergy Biocolor Science & Technology Company). Equal amounts of cell extracts (40 µg) were resolved on 8-10% SDS-PAGE, and transferred into PVDF membranes. The membranes were blocked by 10% non-fat milk at room temperature for 60 min. The following primary antbodieds were used: Anti-ObR, 1:1,000 (cat. no. ab2139; Abcam, Cambridge, UK), Anti-Glut1, 1:1,000 (cat. no. ab115730; Abcam), Anti-Glut4, 1:100 (cat. no. ba1626; Boster, Co., Ltd., Beijing, China) and anti-beta actin, 1:1,000 (cat. no. bs0061R; Bosis Co., Ltd, Beijing, China), at 4˚C overnight. Goat-anti-mouse IgG horseradish peroxidase (HRP)-conjugated secondary antibody (dilution, 1:5,000; cat. no. ZDR-5307, ZSJQ-BIO, Beijing, China) and IgG HRP-conjugated goat-anti-rabbit secondary antibody, (dilution, 1:5,000; cat. no. ZDR-5306, ZSJQ-BIO Beijing, China) were incubated with the membranes at room temperature for 60 min. The proteins detected using a chemiluminescence detection kit (EMD Millipore, Billerica, MA, USA). Western blotting results were analyzed using FluorChem R and AlphaView SA software (version 3.4.0.0) (both from ProteinSimple, San Jose, CA, USA).

RNAi experiment. Leptin receptor specific siRNA and scrambled control siRNA were purchased from GeneChem, Inc. (Daejeon, Korea). The sequence of siRNA: ObR-RNAi-a: 5'-Ccgg, stem gcCTATGAGCAAAGTAAATAT, loop CTCGAG, stem ATATTTACTTTGCTCATAGGC, 3'-TTTTTg; ObR-RNAi-b: 5'aattcaaaaa, stem gcCTATGAG CAAAGTAAATAT, loop CTCGAG, stem ATATTTACTTTG CTCATAGGC. The sequence of control siRNA: TTCTCC GAACGTGTCACGT. MOLT-3 cells were plated in 60 mm culture dish for 24 h prior to transfection. Complete culture medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA), supplemented with FBS, and Penicillin-Streptomycin Solution (cat. no. 15140-122; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) was freshly added to each well 2 h before transfection. Next, the cells were treated using ObR siRNA or scrambled control siRNA (1 µl; dilution, 1:100), the ratio of Nanofectin transfection reagent (ExCell Biology, Inc., Shanghai, China): DNA was 1:1. After 24 h, the transfection efficiency was checked by western blot analysis with ObR expression.

Statistical analysis. Statistical analysis was performed using StataCorp LP 12.0 (College Station, TX, USA). All results are presented as the mean ± standard deviation. Statistical analysis was performed by one-way analysis of variance and Student-Newman-Keuls test. Fisher's exact probability test was used to examine associations between nominal variables. Other statistical analyses of data were performed using the Student's t-test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Immunohistochemical detection and association between ObR and Gluts in patients with TCL. The incidence of ObR expression (Fig. 1) in TCL was 58.3% (21/36), and reduced or absent expression was observed in 41.7% (15/36) of cases. ObR expression was significantly associated with Glut1 (P=0.007), but not with Glut4 (P=0.292). Furthermore, no significant associations were observed between ObR overexpression and age, sex, performance status, Ann Arbor stage (16), lactate dehydrogenase (LDH) levels or B-symptoms (Table I). The expression of ObR was significantly higher in the TCL tissues than in the RLH tissues (P=0.012; Table II).

Expression of ObR and Gluts in human TCL cell lines. Expression of ObR, Glut1 and Glut4 was detected in three TCL cell lines (Jurkat, MOLT-3 and HUT-78) and PBMCs from one

Table I. Association between clinical characteristics or ObR expression and expression of Glut1 and Glut4 in patients with T-cell lymphoma.

No. patients	Total, n	Increased ObR expression, n	Reduced ObR expression, n	P-value
Age				0.223
≤60	13	6	7	
>60	23	15	8	
Sex				0.087
Female	13	10	3	
Male	23	11	12	
ECOG PS				0.673
0-2	24	14	10	
3-4	12	7	5	
Ann Arbor Stage				0.363
I-II	24	13	11	
III-IV	12	8	4	
LDH[a]				0.285
Normal (<250 U/l)	16	8	8	
High (>250 U/l)	20	13	7	
B symptoms[b]				0.456
Yes	20	11	9	
No	16	10	6	
Glut1 expression				0.007
High	17	14	3	
Low	19	7	12	
Glut4				0.292
High	5	4	1	
Low	31	17	14	

[a]LDH categorization is based on the range of normal values in the clinical laboratory at our institution. [b]B symptoms refer to systemic symptoms of fever, night sweats and weight loss. ObR, leptin receptor; ECOG PS, Eastern Cooperative Oncology Group Performance Status; LDH, lactate dehydrogenase.

Table II. ObR expression in TCL and RLH tissues.

	Overexpression of ObR	Reduced expression of ObR	P-value
TCL	21	15	0.012
RLH	4	14	

ObR, leptin receptor; TCL, T-cell lymphoma; RLH, reactive lymphoid hyperplasia.

healthy donor using western blot analysis. As demonstrated in Fig. 2, the protein expression levels of the target proteins varied among the different cell lines. Jurkat and MOLT-3 cells express ObR and Glut1 at higher levels than normal mononuclear cells, while expression differences were not significant between HUT-78 cells and normal control cells. In addition, no significant differences in Glut4 expression between the TCL cells and healthy control cells.

High glucose and leptin increases the proliferation of MOLT-3 cells. The effects of glucose concentration and leptin on the proliferation of MOLT-3 were determined using a CCK-8 assay. MOLT-3 cells were initially serum-starved for 24 h and were then stimulated with different doses of glucose (1,000 mg/l or 4,500 mg/l) and recombinant leptin (0, 10, 100 or 200 ng/ml) for 24, 48, 72 and 96 h. In the first and second 24 h intervals, no significant difference in cell proliferation were identified between the two glucose concentration groups. However, following co-culture for 72 h, the high concentration group exhibited a stronger capacity of proliferation and the trend became even more apparent when cells were treated with glucose for 96 h (Fig. 3A). During the process of co-culture with rhleptin, cell proliferation was revealed to increase along with an increasing concentration of rhleptin, particularly following continuous stimulation for 72 h (Fig. 3B), demonstrated that it was dose- and time-dependent.

Leptin induces glucose uptake in MOLT-3 cells. Glucose uptake from the cell culture medium was measured using the Glucose (HK) assay kit. Stimulation of MOLT-3 cells

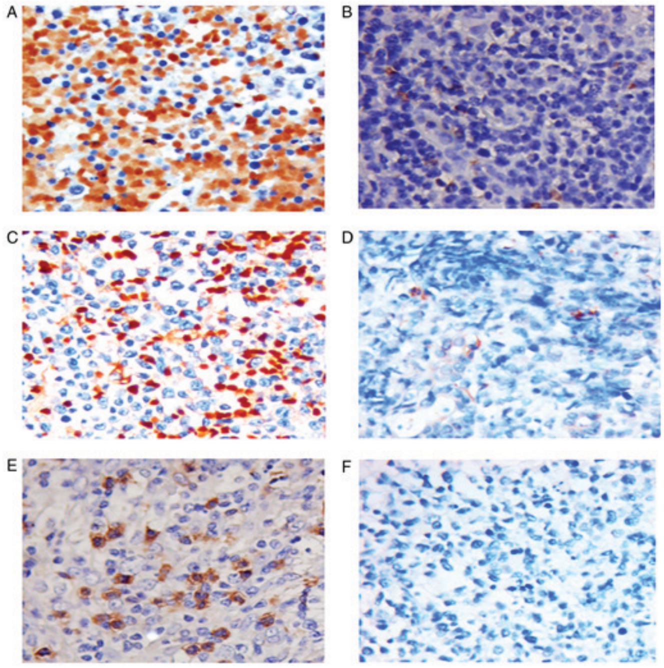

Figure 1. Immunohistochemical staining of ObR, Glut1 and Glut4 in TCL tissues: TCL tissue samples exhibiting (A) high expression of ObR or (B) low expression of ObR; (C) high expression of Glut1 or (D) low expression of Glut1; (E) high expression of Glut4 or (F) low expression of Glut4. Magnification, x400. ObR, leptin receptor; Glut, glucose transporter; TCL, T-cell lymphoma.

treated with rhleptin induced a dose-dependent increase in the uptake of glucose after 30 min of incubation (Fig. 4A). Additionally, after 48 h stimulation with rhleptin, the maximal glucose uptake was observed at a concentration of 100 ng/ml (Fig. 4B), which may be associated with the Glut expression and transportation induced by leptin pathway.

Leptin induces recruitment of Glut1 to the cell surface. Following short-term incubation with 100 ng/ml rhleptin, the amount of Glut1 present changed over time. The effect of leptin increased to its peak at 30 min and declined gradually thereafter. While no significant changes in ObR or Glut4 were detected under the same conditions (Fig. 5A and C).

Leptin increases the expression of Glut1 mRNA. To further investigate whether the leptin-induced glucose uptake is dependent on the overexpression of Gluts, the effect of leptin on the mRNA levels of ObR, Glut1 and Glut4 genes was measured by RT-qPCR. Following treatment with 100 ng/ml rhleptin for 48 h, the expression of Glut1 mRNA in MOLT-3 cells was upregulated over 2-fold, while no significant difference in Glut4 mRNA expression was observed between the rhleptin-treated and the untreated cells. In addition, leptin did not appear to affect the expression of ObR (Fig. 5E).

Leptin upregulates Glut1 protein expression. Whether the protein expression levels of ObR, Glut1 and Glut4 were altered in MOLT-3 cells following rhleptin treatment was also investigated. As demonstrated in Fig. 5B and D, following treatment with different doses of leptin (0, 10, 100 or 200 ng/ml) for 48 h, the Glut1 expression was markedly upregulated, while no considerable changes in ObR and Glut4 expression were observed under the same conditions.

Effect of ObR inhibition with siRNA on the glucose uptake of MOLT-3 cells. siRNA for ObR was transfected into MOLT-3 cells, which notably decreased ObR expression and blocked leptin-induced Glut1 expression, but did not change the expression of Glut4 (Fig. 6A). Transfection with specific siRNAs did not affect the expression levels of unrelated genes, including β-actin. As demonstrated in Fig. 6B, ObR-specific siRNA reduced the glucose uptake of MOLT-3 cells.

Discussion

The present study demonstrated that leptin and its receptor significantly increased glucose uptake in MOLT-3 cells. In addition, it was revealed that the mechanism by which leptin induced glucose uptake was through the increased Glut1 expression and transportation to the cell surface. Therefore, to the best of our knowledge, the present study is the first to discuss the glucose consumption promoting effect of leptin on TCL cells *in vitro*.

The lepin/leptin receptor signaling pathway is well-known to take part in energy metabolism, cell proliferation and immunomodulation (16). Previous studies have proven the role of leptin and its receptor in the occurrence and progression of cancer (17). These observations are further supported by experimental evidence that leptin may stimulate proliferation and inhibit apoptosis in different types of cancer cell (18). However, the direct effect of the leptin/leptin receptor signaling pathway on glucose metabolism in malignant cells has been rarely studied. Therefore, the present study investigated the effect of leptin on the glucose uptake of malignant T cells and examined the mechanism involved in the process.

It is widely believed that leptin exerts direct effects on glucose levels independently of body weight and food intake. In the central nervous system, leptin and insulin share a similar phosphoinositide 3-kinase (PI3K) intracellular signaling pathway to modulate glucose uptake and metabolism (19). At the peripheral level, this role for leptin has been established in metabolic cells, including muscle and adipose cells (20). With regards to T cells, leptin upregulates glucose uptake and Glut1 expression through mechanistic target of rapamycin signaling in activated T cells compared with that in resting T cells (21). We hypothesized that malignant T cells/TCL tissues may express a higher level of ObR than normal mononuclear cells/RLH tissues, and that leptin/leptin receptor signaling may promote glucose uptake in malignant T cells.

As expected, the present study observed a higher level of ObR expression in TCL pathological sections than in RLH tissues (58.3 vs. 22.2%; P=0.012). Additionally, the overexpression of ObR was associated with Glut1 expression in TCL (P=0.007). In vitro, TCL Jurkat and MOLT-3 cells exhibited upregulated ObR expression, which was not the case for the cutaneous TCL Hut-78 cell line. Previous studies have suggested that this may be partly due to the various sources and activity of TCL cells (22-24). Compared with resting $CD4^+$ T cells, activated $CD4^+$ T cells exhibited a higher expression level of ObR (25). Following lymphocyte activation, ObR expression shifted to a higher intensity and density (26). ObR expression, particularly that of the long

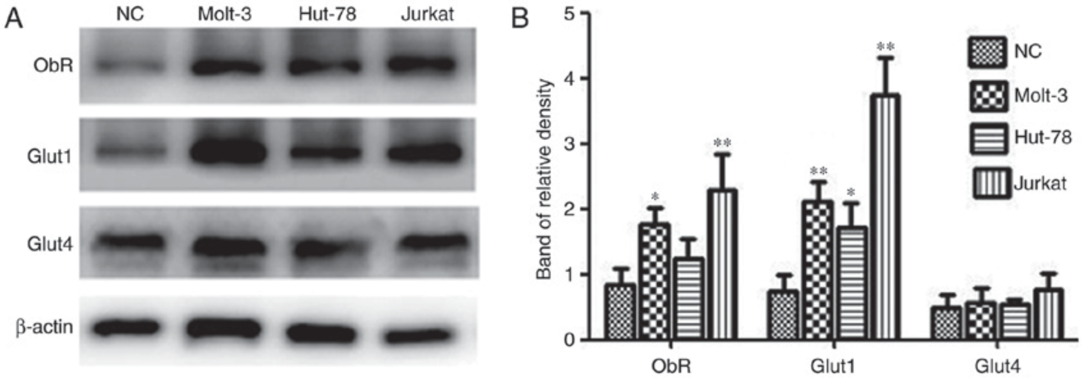

Figure 2. ObR, Glut1 and Glut4 expression in TCL cell lines and PBMCs. (A) Western blotting was used to analyze total protein extracts for ObR, Glut1 and Glut4 expression in TCL cell lines and PBMCs from a healthy donor. β-actin expression was used as an internal control. (B) The relative densities of ObR, Glut1 and Glut4 were calculated from 3 independent experiments. *P<0.05, **P<0.01 compared with ObR, Glut1 and Glut4 expression in PBMCs. NC, normal cells; ObR, leptin receptor; Glut, glucose transporter; TCL, T-cell lymphoma; PBMCs, peripheral blood mononuclear cells.

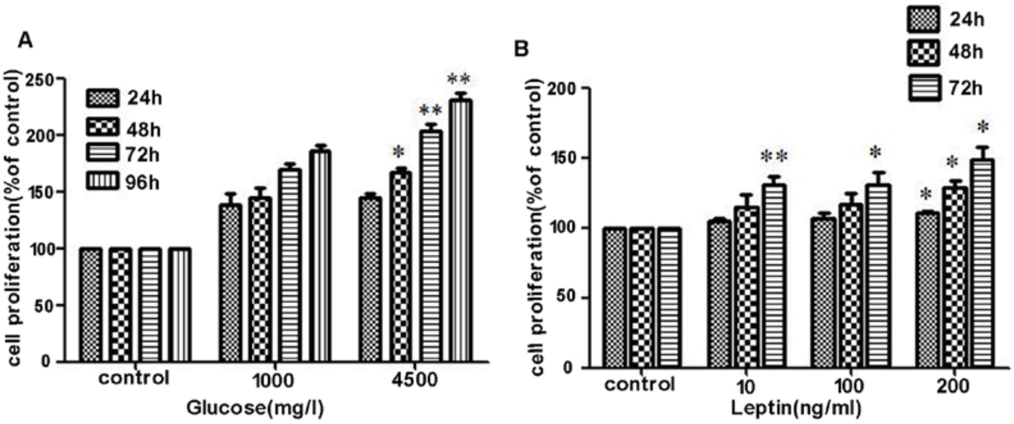

Figure 3. Effects of high glucose and leptin on the proliferation of MOLT-3 cells. (A) MOLT-3 cells were initially serum-starved for 24 h and were then stimulated with different doses of glucose (1,000 mg/l or 4,500 mg/l) and the cell proliferation was determined using a Cell Counting kit-8 assay. Compared with 1,000 mg/l glucose, 4,500 mg/l glucose promoted cell proliferation more markedly. (B) Following treatment with recombinant leptin (0, 10, 100 or 200 ng/ml) for different time periods 24, 48 and 72 h, increased cell proliferation was revealed to be time- and dose-dependent. *P<0.05, **P<0.01. OD, optical density.

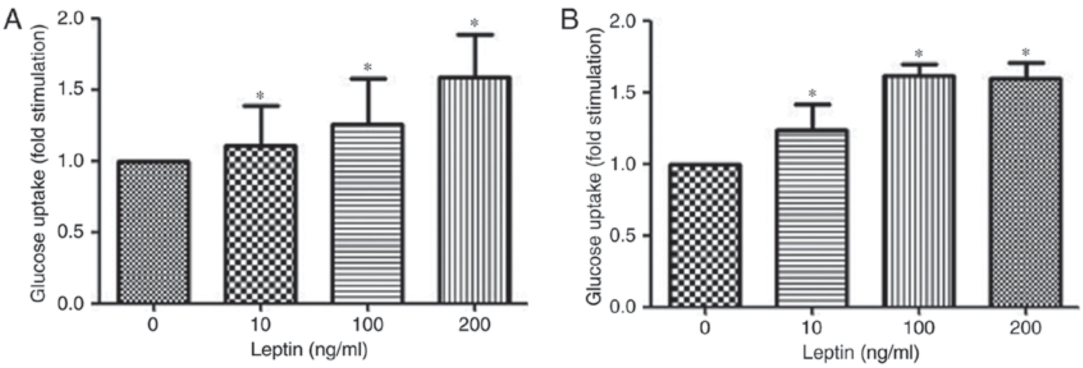

Figure 4. Effect of leptin on the glucose uptake in MOLT-3 cells. (A) MOLT-3 cells were cultured with leptin for 30 min and a dose-dependent increase in glucose uptake was subsequently observed. (B) Stimulation of MOLT-3 cells with leptin induced an increase in the uptake of glucose after 48 h co-culture and the most significant trends were observed at a concentration of 100 ng/ml. Glucose uptake was measured using the Glucose (HK) assay kit. *P<0.05.

isoform, in normal murine thymocytes is exceedingly low or negative (27). However, the present study revealed that Jurkat and MOLT-3 cells, which are suspected to develop from thymocytes or thymic progenitors, exhibit a high expression level of ObR. This may be due to mutation of the upstream regulation sequence or the leptin receptor (LEPR) gene. In the visceral fat of obese patients, a negative correlation between microRNA-145 and LEPR gene expression has been confirmed by Saucillo *et al* (28). Another study identified 26 single nucleotide polymorphisms (SNPs) mapping to the

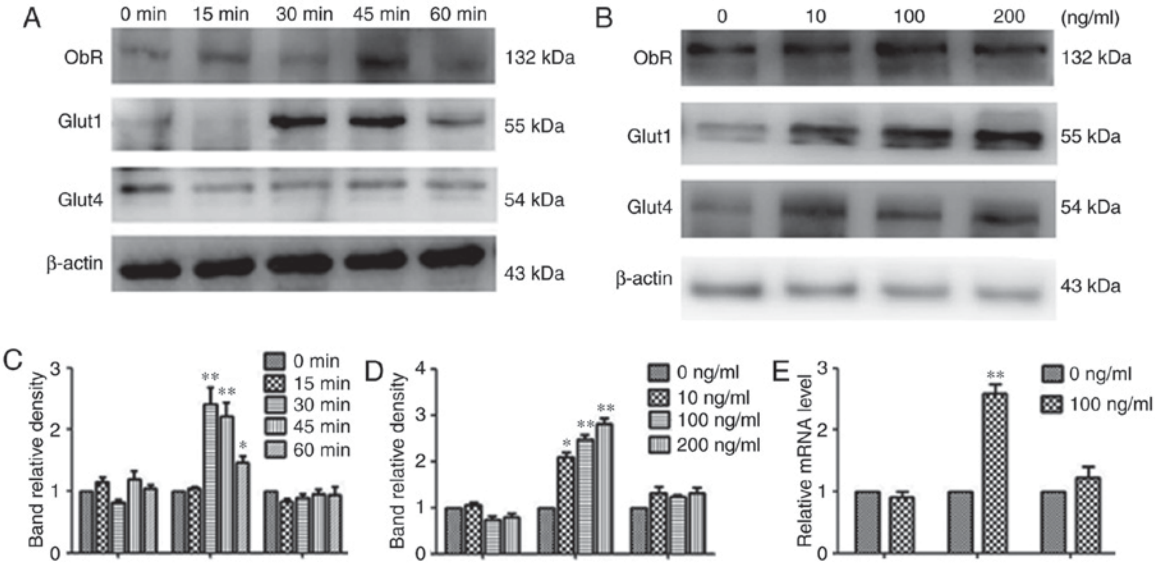

Figure 5. Effects of leptin on the recruitment of Glut1 to the cell surface and effects of leptin on Glut1 and Glut4 in MOLT-3 cells. (A and B) MOLT-3 cells were treated with 100 ng/ml leptin for 0, 15, 30, 45 or 60 min. Next, the cells were collected and the cell membrane proteins were extracted. Western blotting was used to analyze the membrane protein level of ObR, Glut1 and Glut4. β-actin expression was used as an internal control. The relative densities of ObR, Glut1 and Glut4 were calculated from 3 separate experiments. *$P<0.05$, **$P<0.01$. (C and D) Following treatment with different doses of leptin (0, 10, 100 or 200 ng/ml) for 48 h, the protein expression of ObR, Glut1 and Glut4 was measured by western blot analysis and results demonstrated that Glut1 expression was markedly upregulated. *$P<0.05$, **$P<0.01$. (E) Following treatment with 100 ng/ml rhleptin for 48 h, the expression of ObR, Glut1 and Glut4 mRNA in MOLT-3 cells was detected by reverse transcription-quantitative polymerase chain reaction. It was also revealed that Glut1 mRNA expression was upregulated by over 2-fold **$P<0.01$. ObR, leptin receptor; Glut, glucose transporter.

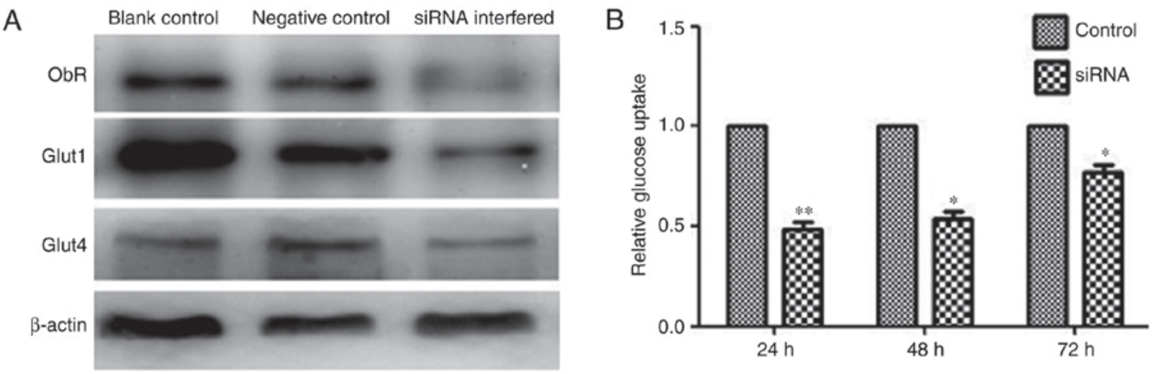

Figure 6. Effect of ObR inhibition with siRNA on the glucose uptake and Glut1 expression in MOLT-3 cells. siRNA for ObR was transfected into MOLT-3 cells. (A) ObR and Glut1 protein expression was notably decreased following transfection. (B) Cell glucose uptake in MOLT-3 cells was significantly inhibited following blocking of the leptin signaling pathway. *$P<0.05$, **$P<0.01$. ObR, leptin receptor; siRNA, small interfering RNA; Glut, glucose transporter.

LEPR region on chromosome 1p31, and revealed that SNP rs12062820 was most strongly associated with plasma soluble leptin receptor expression levels (29). The correlation between genetic characteristics and LEPR expression in malignancies is rarely reported and therefore, requires elucidation in further investigations.

In glucose metabolism regulation, leptin and its receptor have different functions in various tissues and cells by numerous mechanisms. In mouse muscle C2C12 cells, leptin increased glucose uptake, and Glut4, but not Glut1, was recruited to the cell surface by stimulating the signal transducer and activator of transcription 3 (STAT3) and mitogen-activated protein kinase 1 signaling pathways (30). In HepG2 cells, leptin inhibited insulin-stimulated insulin receptor substrate 1 tyrosine phosphorylation, thereby impairing insulin action in the liver, leading to elevated hepatic glucose output (31). In recent years, there has been an increasing amount of interest regarding the metabolism of immune cells. The ability of activated T cells to meet their metabolic requirements depends on glucose import through Gluts, as they do not store large quantities of glycogen (32). The majority of activated T cells take up glucose via Glut1 instead of Glut4, due to the fact that mature T cells primarily express undetectable Glut4 (33). The present study revealed that, in MOLT-3 cells, leptin/leptin receptor signaling modulates glucose uptake in a similar manner as in activated T cells. Stimulation with leptin led to a dose-dependent increase in glucose uptake, which may be associated with the translocation of Glut1 to the cell surface. A 48 h coculture with leptin also promoted the uptake of glucose, and upregulated Glut1 expression was dosage independent. This indicated that the experimental dose of 100 ng/ml almost reached the concentration for a maximal effect and thus, that continuing to increase the concentration would not enhance the effect any further. When the leptin/leptin receptor pathway was interrupted by siRNA, Glut1 expression and glucose uptake were interfered.

The ability of leptin to promote glucose uptake may subsequently lead to increased cellular activities. In previous studies, it has been proven leptin may promote the proliferation of diffuse large B-cell lymphoma and acute myeloid leukemia cells directly via the PI3K/Akt and STAT3 signaling pathways (34-36). Similar to the proliferation of DLBCL and AML cells mentioned in the above studies, promotion activity of leptin was also observed in MOLT-3 cells by CCK8 analysis in the present study, and it was revealed that leptin affected cell proliferation indirectly by the glucose promoting effect, in addition to the direct effect.

In summary, TCL consists of a group of diseases lacking effective treatments and associated with a poor prognosis. The study of targeted therapy for TCL remains a challenge. The results of the present study suggested that leptin and its receptor participate in the glucose metabolism of TCL cells by upregulating the expression and recruitment of Glut1. Therefore, blocking of the leptin/leptin receptor pathway may be useful as a potential therapeutic strategy against TCL and further study is required to confirm this.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

HZX and XW conceived and designed the study. TJH and JSL performed the experiments. TJH and XYL wrote the paper. XYL and XXZ analysed data. LYG was involved in data collection. All authors read and approved the final manuscript.

Ethics statement and consent to participate

The present study was conducted with the approval of the Ethics Committee of Tai'an Central Hospital (Tai'an, China) and written informed consent was obtained from all participants.

Patient consent for publication

Written informed consent was received from all participants for the publication of this study.

Competing interests

The authors declare that they have no competing interests.

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