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Leptocarpin Suppresses Proliferation, Migration, and Invasion of Human Osteosarcoma by Targeting Type-1 Insulin-Like Growth Factor Receptor (IGF-1R)

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

BCDEF 1 Shao-wei Zheng*
BCDEF 2 Wen-guo Wan*
BCDEF 1 Hai-xiong Miao*
BCDEF 3 Rui Tang*
BCDEF 1 Bin Wang*
BCDEF 1 Qi-zhi Huang*
BCDEF 1 Wei-le Liu
BCDEF 1 Jian-ping Zheng
BCDEF 1 Chu-qun Chen
BCDEF 1 Hao-bo Zhong
AG 1 Sheng-fa Li
AG 1 Chun-han Sun

1 Department of Orthopedics, Huizhou Medical Research Center, Huizhou 1st People's Hospital, Huizhou, Guangdong, P.R. China
2 Department of Orthopedics, Longmen People's Hospital, Huizhou, Guangdong, P.R. China
3 Department of Cell Biology, Southern Medical University, Guangzhou, Guangdong, P.R. China

* Shao-wei Zheng, Wen-guo Wan, Hai-xiong Miao, Rui Tang, Bin Wang, and Qi-zhi Huang contributed equally

Corresponding Authors: Sheng-fa Li, e-mail: lishengfahz@163.com and Chun-han Sun, e-mail: sunchunhanhz@163.com

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Background: Leptocarpin (LTC) has drawn much attention for suppressing tumor growth or reducing inflammation. However, the effect of LTC on osteosarcoma has rarely been reported. Our object was to determine whether LTC suppresses MG63 cell proliferation, migration, and invasion, and whether type-1 insulin-like growth factor receptor (IGF-1R) is one of the targets in LTC suppressing osteosarcoma.

Material/Methods: Cytotoxicity of LTC was performed by use of a cell-counting kit-8 (CCK-8). RNA interference (RNAi) or pEABE-bleo IGF-1R plasmid were used for silencing or overexpressing IGF-1R, Western blot (WB) analysis was used for IGF-1R expression, CCK-8 for proliferation, and transwell assay for migration and invasion.

Results: LTC (23.533 μM) treatment for 48 h was taken as the 50% inhibiting concentration (IC50), which significantly ($P<0.05$) suppressed MG63 cells proliferation, migration, and invasion. LTC (IC50) obviously inhibited IGF-1R expression in MG63 cells, with similar effect to small interfering RNA (siRNA), while pEABE-bleo IGF-1R transfection overexpressed IGF-1R. siRNA silencing IGF-1R suppressed MG63 cells proliferation, migration, and invasion, while pEABE-bleo IGF-1R transfection was significantly ($P<0.05$) promoted. With or without siRNA or pEABE-bleo IGF-1R transfection, LTC (IC50) suppressed MG63 cells proliferation, migration, and invasion. The effect of LTC (IC50) combined with siRNA on suppressing MG63 cells proliferation, migration, and invasion was more obvious, while the effect of LTC (IC50) combined with pEABE-bleo IGF-1R transfection was less significant ($P<0.05$).

Conclusions: LTC suppressed osteosarcoma proliferation, migration, and invasion by inhibiting IGF-1R expression. IGF-1R is one of the targets in LTC suppressing osteosarcoma.

MeSH Keywords: Cell Migration Inhibition • Cell Proliferation • Osteosarcoma

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Background

Bone is a dynamic tissue with a rigid and mineralized extracellular matrix. It undergoes constant remodeling, which is determined by the effect of osteoclast activity [1]. During this process, old and damaged bone and osteoblasts are resorbed, while new mineralized extracellular matrix is synthesized [2,3]. Bone remodeling plays an important role in maintaining structure throughout life, with an intimate interaction between osteoclastic and osteoblastic activities [3]. The imbalance between bone resorption and synthesis leads to loss of bone integrity and function, as well as bone metabolic disorders [4].

Osteosarcoma, involving the formation of malignant osteoid or woven bone, is one of the most common tumors clinically, which frequently affects children and young adults [5]. Osteosarcoma is related to high rates of bone destruction with great biological virulence [6]. However, further attentions should be on the metastases and invasion of osteoclastic cells, even with osteosarcoma, clinically. According to a previous report [6], tumor-associated osteoclasts were found in 63% of patients by histological and pathological detections, as well as in 75% by mRNA analysis. This indicates that there is a close relationship between osteoclastic activity and osteosarcoma aggressiveness. In our research, MG63 cells were used to investigate the effect of LTC on migration, invasion, and proliferation.

In the clinical treatment of neoplasia, chemotherapeutic drugs, which may be natural products or chemical derivatives, are used increasingly and widely [7]. Recently, the research on new products with potential biological activity has been focused on medicinal plants. Asteraceae, which produces many bioactive compounds such as polyacetylenes, diterpenes, and sesquiterpene lactones [8], expresses various clinical activities, including anti-tumor, anti-ulcer, anti-inflammatory, neurocytotoxic, and cardiotonic activities [9,10], as well as the ability to influence critical biological processes such as cell signaling, cell proliferation, apoptosis, and mitochondrial metabolism [11]. Leptocarpin (LTC) is the one of the major components of *Leptocarpha rivularis*, which belongs to the Asteraceae family and was been widely used as a folk remedy [12–14]. In our study, LTC was used as an anti-tumor drug to treat MG63 cells.

At the genetic level, previous research [15] has shown that many genetic and epigenetic agents are involved in tumor pathogenesis and progression. According to Liu et al. [16], PMP22 (peripheral myelin protein 22) was found to be overexpressed in osteosarcoma. The *in vivo* experiments proved the relationship between PMP22 and the proliferation, migration, invasion, and colony formation of osteosarcoma cells. In addition, Actein was found to inhibit osteosarcoma proliferation and migration by Chen et al. [17]. Burguera et al. [18] studied the role of leptin in human osteosarcoma cells, and they found that leptin promoted osteosarcoma cell proliferation, which was related to the activation of PI(3)-K and MAPK pathways. All of these results suggest that many molecules play important roles in tumor proliferation and migration.

IGF-1R is a member of the tyrosine protein kinase receptor family. It participates in the establishment of a malignant cell phenotype [19], cell metastasis [20], protection from apoptosis [21], and enhancement of cell proliferation [22]. According to Hirano et al. [23], high level of IGF-1R expression, as the critical prognostic factor, was correlated to tumor progression in human endometrial carcinoma. Pavelic et al. [24] also found that endometrial cancer cells synthesized and secreted IGF-I and IGF-II, integrating with IGF-1R and inducing tumor proliferation. Although IGF-1R is highly overexpressed in most malignant tissues, where it functions as an anti-apoptotic agent by enhancing cell survival, whether IGF-1R could be used as a molecular target in suppressing osteosarcoma growth has been unknown.

Here, we used RNAi to silence *IGF-1R* gene expression to investigate the role of IGF-1R in LTC suppressing MG63 cell proliferation, migration, and invasion.

Material and Methods

MG63 cell

MG63 cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA) and cultured in Dulbecco's modified Eagle's medium (DMEM, Thermo Fisher Scientific Inc., Shanghai, China) containing 10% fetal bovine serum (FBS, Gibco, Thermo, Shanghai, China), amphotericin (2.5 μg/mL, Sigma-Aldrich Inc., Shanghai, China), penicillin (100 U/mL, Sigma-Aldrich), and streptomycin (100 μg/mL, Sigma-Aldrich) under conditions of 5% CO_2, 37°C, and saturated humidity. When 90% confluent, the cells were digested with 0.25% trypsin-EDTA (Thermo) and subcultured.

LTC preparation

LTC was extracted from *Leptocarpha rivularis* according to previous methods [25] and sent to the Scistd Testing Institute (Qingdao, China) for structural identification by spectroscopic techniques (^1H and ^{13}C NMR, IR, MS). LTC was dissolved in dimethylsulfoxide (DMSO, Sigma-Aldrich), with primary concentration adjusting as 1 mg/mL and stored at –20°C. Before use, LTC (1 mg/mL) was diluted with medium as given concentrations from 1.0 to 25.0 μM.

LTC cytotoxicity screening in MG63 cells by CCK-8

MG63 cells were digested and the concentration was adjusted to 3000 cells in 200 μL medium per well in a 96-well plate.

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After culturing for 24 h, MG63 cells were treated with LTC (1.0, 2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5, 20.0, 22.,5 and 25.0 μM). The negative control (NC) group was the MG63 cells treated with 0.1% DMSO. All the cells were incubated at 37°C, with 5% CO_2 and saturated humidity, for 24, 48, and 72 h. After treatments, 10 μl of CCK-8 buffer was added to each well. The cells were detected at 450 nm by an enzyme mark instrument (Synergy HTX multi-mode reader, BioTek Instruments, Co. Ltd., USA) after 20 min. The data obtained are shown as percentages of living cells versus the control, expressed as mean ± standard deviation (SD).

Silencing IGF-1R

siRNA targeting IGF-1R (5'-GCC GAT GTG TGA GA AGC-3') was synthesized by Shanghai GenePharma Co., Ltd. (Shanghai, China). IGF-1R siRNA was used to transfect MG63 cells with Lipofectamine® 3000 reagent (Thermo) according to the specifications. MG63 cells were cultured subsequently for 72 h. Then, LTC was used to treat MG63 cells and were compared to NC (0.1% DMSO).

Overexpressing IGF-1R

pEABE-bleo IGF-1R plasmid was obtained from Addgene (Beijing Zhongyuan, Ltd. China). MG63 cells were transfected with pEABE-bleo IGF-1R plasmid by Lipofectamine® 3000 reagent for 48 h and then treated with LTC and compared to NC (0.1% DMSO).

Detection on MG63 cell migration and invasion

For detection of MG63 invasion, 5 μl Matrigel (Becton, Dickinson and Company, BD, USA) was spread in the upper chamber of a transwell 24-well plate (BD). Following treatment with LTC for 48 h or 72 h, MG63 cells were cultured with DMEM without FBS or antibiotics for 6 h, and then digested as density of 1×10^5 cells/mL with DMEM without FBS. We added 0.5 mL of the cell suspension to each upper chamber of the 24-well plate, while 0.5 mL DMEM containing 10% FBS was added to the lower chamber. The upper chamber was placed in the appropriate lower chamber. After 24 h, the Matrigel and the MG63 cells in the upper chambers were cleaned carefully and thoroughly. The upper and lower chambers were washed once with 1×PBS and the cells were fixed with 4% paraformaldehyde (Solarbio Science and Technology Co., Ltd. Beijing China) for 20 min. MG63 cells in the lower surface of the upper chambers were stained with 0.25% crystal violet (Macklin Inc. Shanghai, China), which was dissolved in 20% methanol, for 20–45 min. The samples were washed twice with PBS and the membrane of the chamber was sealed with cover balsam (Leica Biosystems, Germany). The membrane was wetted with PBS and observed under a microscope (Nikon, Japan). Five fields (20×) were selected randomly and the number of MG63 cells was counted, with the average number and the comparisons analysis.

For detection MG63 cell migration, the protocol was the same as for invasion, but without Matrigel.

Detections of IGF-1R expression

Total proteins in MG63 cells were extracted with loading buffer (2×) and the concentrations were determined with a BCA protein assay kit (Beyotime Biotechnology Company, Shanghai, China) according to the specifications. The proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with a 10% separating gel and 5% stacking gel. Then, the protein was transferred to polyvinylidene fluoride (PVDF) membranes (Merck Millipore Corporation, Darmstadt, Germany), and the membranes were blocked with 5% non-fat milk and 2% bovine serum albumin (BSA, Gen-View Scientific Inc., USA) in PBS-T (PBS containing 0.25% Tween-20, Macklin) at 25°C for 1.5 h. The membranes were incubated with rabbit anti-IGF-1R primary antibodies (1: 4000, Proteintech Group Inc., Wuhan, China) and mouse anti-β-actin (1: 10 000, Proteintech), as internal control, at 4°C overnight. The next day, the membranes were incubated with goat anti-rabbit and goat anti-mouse secondary antibodies (1: 3000, Jackson ImmunoResearch Laboratories, Inc. USA) at 25°C for 2 h, and then analyzed with electrochemiluminescence (ECL, Millipore, MA, USA).

Statistical analysis

All the data were analyzed using SPSS 19.0 software. The data are shown as mean ± standard deviation (X±SD). Cell proliferation is shown as optical density (OD) value. Cell migration and invasion are shown as cell amount. One-way ANOVA was used for comparisons among all treatments. IC50 was analyzed by probit regression. Least significant difference (LSD) was used for multiple comparisons when there was homogeneity of variance, while Dunnett's T3 test was used with heterogeneity of variance. $P<0.05$ was considered to indicate a significant difference.

Results

LTC suppressing MG63 cells proliferation

The cytotoxicity of LTC is shown in Table 1 and Figure 1A. There was no significant difference in MG63 proliferation with different LTC concentrations treatment for 24 h. Treating for 48 h, the proliferation of MG63 cells treated with LTC (10.0~25 μM) were both significantly ($P<0.05$) lower than the NC. The proliferation of MG63 cells treated with LTC (7.5~25 μM) for 72 h

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Table 1. Effect of 0.1% DMSO or LTC (1.0~25μM) on MG63 cells proliferation in 24 h, 48 h and 72h (X±SDs).

	Time		
	24 h	48 h	72 h
NC (0.1% DMSO)	0.176±0.034	0.510±0.079	0.986±0.047
LTC (1.0 μM)	0.179±0.008	0.506±0.075	0.924±0.068
LTC (2.5 μM)	0.168±0.016	0.504±0.042	0.910±0.094
LTC (5.0 μM)	0.159±0.019	0.505±0.086	0.782±0.087
LTC (7.5 μM)	0.162±0.025	0.492±0.071	0.706±0.071[1]
LTC (10.0 μM)	0.156±0.018	0.451±0.027[2]	0.685±0.069[1]
LTC (12.5 μM)	0.155±0.037	0.427±0.016[2]	0.595±0.043[1]
LTC (15.0 μM)	0.150±0.054	0.387±0.060[2]	0.510±0.036[1]
LTC (17.5 μM)	0.145±0.008	0.355±0.200[2]	0.428±0.029[1]
LTC (20.0 μM)	0.149±0.017	0.301±0.016[2]	0.353±0.053[1]
LTC (22.5 μM)	0.140±0.018	0.285±0.074[2]	0.332±0.078[1]
LTC (25.0 μM)	0.137±0.021	0.265±0.043[2]	0.306±0.071[1]

DMSO – dimethylsulfoxide; LTC – Leptocarpin; NC – negative control group; [1] comparing to the NC (72h), $P<0.05$; [2] comparing to the NC (48h), $P<0.05$

were significantly ($P<0.05$) lower than the NC. The IC50 was 23.533 μM LTC (95% CI=22.269~25.088) treated for 48 h or 16.548 μM LTC (95% CI=15.017~18.345) treated for 72 h.

Therefore, two IC50 values were obtained, 23.533 μM LTC treated for 48 h and 16.548 μM LTC treated for 72 h. We chose 23.533 μM LTC treated for 48 h as the final IC50 in the following treatments.

LTC inhibits IGF-1R expression in MG63 cells

As shown in Figure 1B, siRNA or 23.533 μM LTC treated for 48 h decreased IGF-1R expression in MG63 cells, while pEA-BE-bleo IGF-1R clearly increased IGF-1R expression, indicating the successfully overexpression or silencing of IGF-1R. After overexpression of IGF-1R, 23.533 μM LTC treatment for 48 h obviously decreased IGF-1R in MG63 cells compared to 0.1% DMSO treatment. On the other hand, after silencing IGF-1R, 23.533 μM LTC treatment for 48 h further decreased IGF-1R in MG63 cells compared to 0.1% DMSO or LTC treatment alone.

LTC suppresses MG63 cells migration and invasion

The results of migration and invasion of MG63 cells are shown in Table 2, Figure 1C, and Figure 1D. There were significant ($P<0.05$) differences in migration (Figure 1Ca, 1Cb) and invasion (Figure 1Da, 1Db) of MG63 cells treated with 23.533 μM LTC for 48 h.

LTC suppresses MG63 cell proliferation

As shown in Table 3, Figures 2A and 2B, we compared the proliferation rate of MG63 cells with different treatments. MG63 cells proliferation rate was promoted after overexpressing IGF-1R, but suppressed after silencing IGF-1R, which indicates that overexpressed IGF-1R promotes MG63 cells proliferation. LTC 23.533 μM treatment for 48 h obviously suppressed MG63 cell proliferation, similar to RNAi. After overexpressing IGF-1R, 23.533 μM LTC treatment for 48 h suppressed MG63 cell proliferation compared to the NC group. Additionally, siRNA silencing IGF-1R combined with 23.533 μM LTC treatment for 48 h further suppressed MG63 cell proliferation compared to siRNA or LTC treatment alone.

LTC suppresses MG63 cell migration and invasion

As shown in Table 4 and Figure 2C–2F, we compared the migration and invasion of MG63 cells with different treatments. MG63 cell migration and invasion rates were both promoted after overexpressing IGF-1R, but were suppressed after silencing IGF-1R or 23.533 μM LTC treatment for 48 h, compared to NC. These results indicate that overexpressed IGF-1R promotes MG63 cell migration and invasion. After overexpressing IGF-1R, 23.533 μM LTC treatment for 48 h still suppressed MG63 cell migration and invasion compared to NC. LTC 23.533 μM treatment for 48 h after siRNA silencing of IGF-1R further suppressed MG63 cell migration and invasion, with better effects than siRNA or LTC treatment alone.

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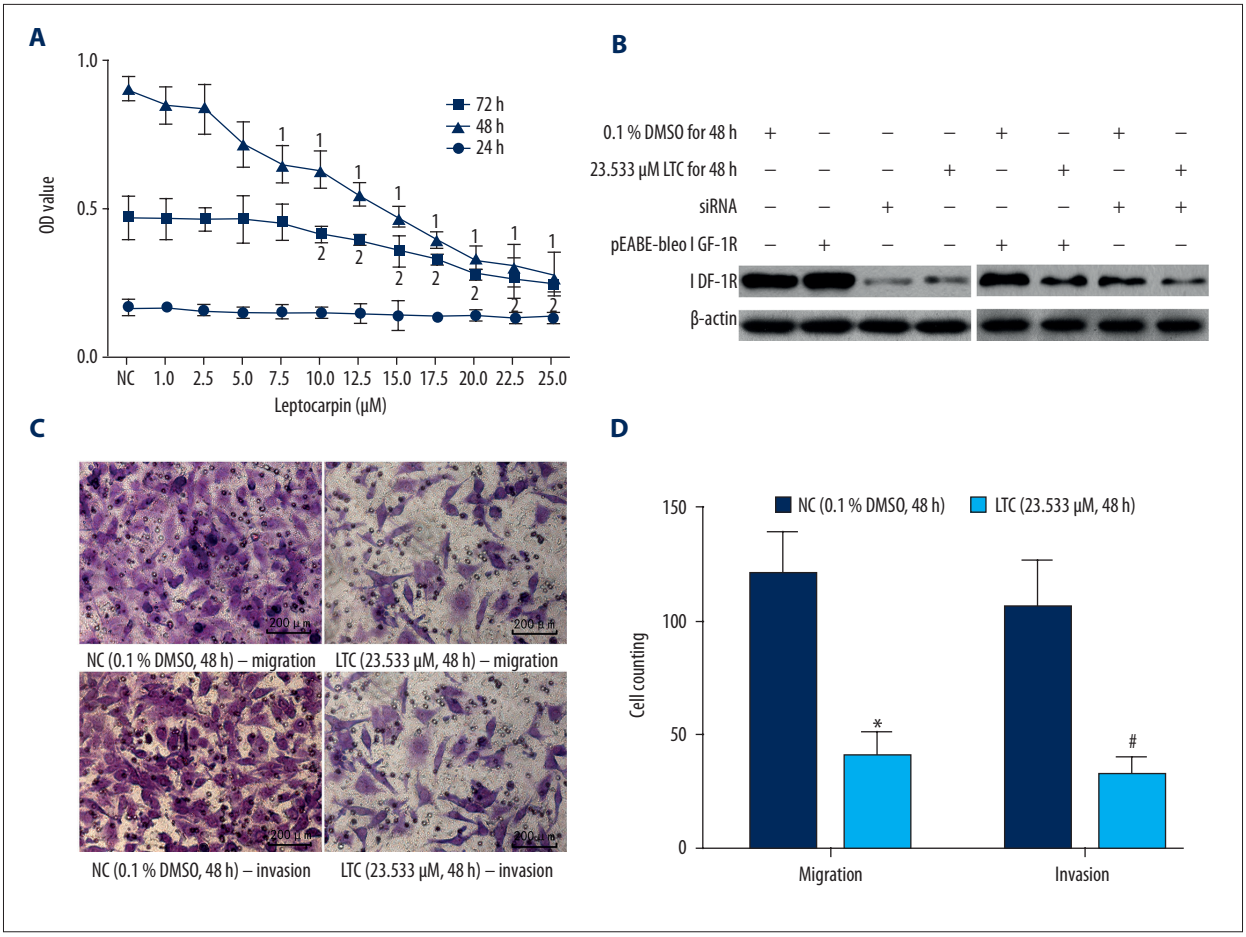

Figure 1. Effect of LTC on IGF-1R and MG63 cell proliferation, migration, and invasion. (**A**) Effect of 0.1% DMSO or LTC on MG63 cells proliferation at 24, 48 and 72 h ([1] compared to NC (72 h), $P<0.05$; [2] compared to NC (48 h), $P<0.05$). (**B**) Effect of LTC on IGF-1R expression in MG63 cells (β-actin: internal reference). (**C**) Effect of LTC on MG63 cell migration and invasion. (**D**) Effect of LTC on MG63 cell migration and invasion rates ([1] compared to migration in NC, $P<0.05$; [2] compared to migration in NC, $P<0.05$).

Table 2. Effect of 0.1% DMSO or LTC on MG63 cells migration and invasion (X±SDs).

	Migration	Migration rate (%)	Invasion	Invasion rate (%)
NC (0.1% DMSO, 48 h)	120.68±18.04	–	106.36±20.59	–
LTC (23.533 μM, 48 h)	40.56±10.31[1]	33.61	32.12±8.43[2]	30.20

LTC – Leptocarpin group; NC – negative control group; [1] comparing to the NC (0.1% DMSO, 48 h) of migration, $P<0.05$; [2] comparing to the NC (0.1% DMSO, 48 h) of invasion, $P<0.05$

Discussion

Osteosarcoma, a malignant bone tumor, affects many children and young adults worldwide. To find new anti-tumor drugs and targets for the treatment of osteosarcoma, we chose MG63 cells as the subject and used LTC as the anti-tumor drug. By silencing IGF-1R with RNAi, we found that IGF-1R was one of the targets in LTC's suppression of MG63 cell proliferation, invasion, and migration.

LTC is a sesquiterpene lactone, a major component of the Asteraceae family [25]. According to Martinez et al. [12], LTC exhibits high cytotoxicity on human liver adenocarcinoma (SK-Hep-1) and HeLa cells. Therefore, LTC has anti-cancer and anti-tumor effects [26–28]. However, there is less study focusing on the effect of LTC on osteosarcoma. In our investigations, we studied the cytotoxicity of LTC on MG63 cells, and we found that LTC (10.0~25 μM) treatment for 48 h and LTC (7.5~25 μM)

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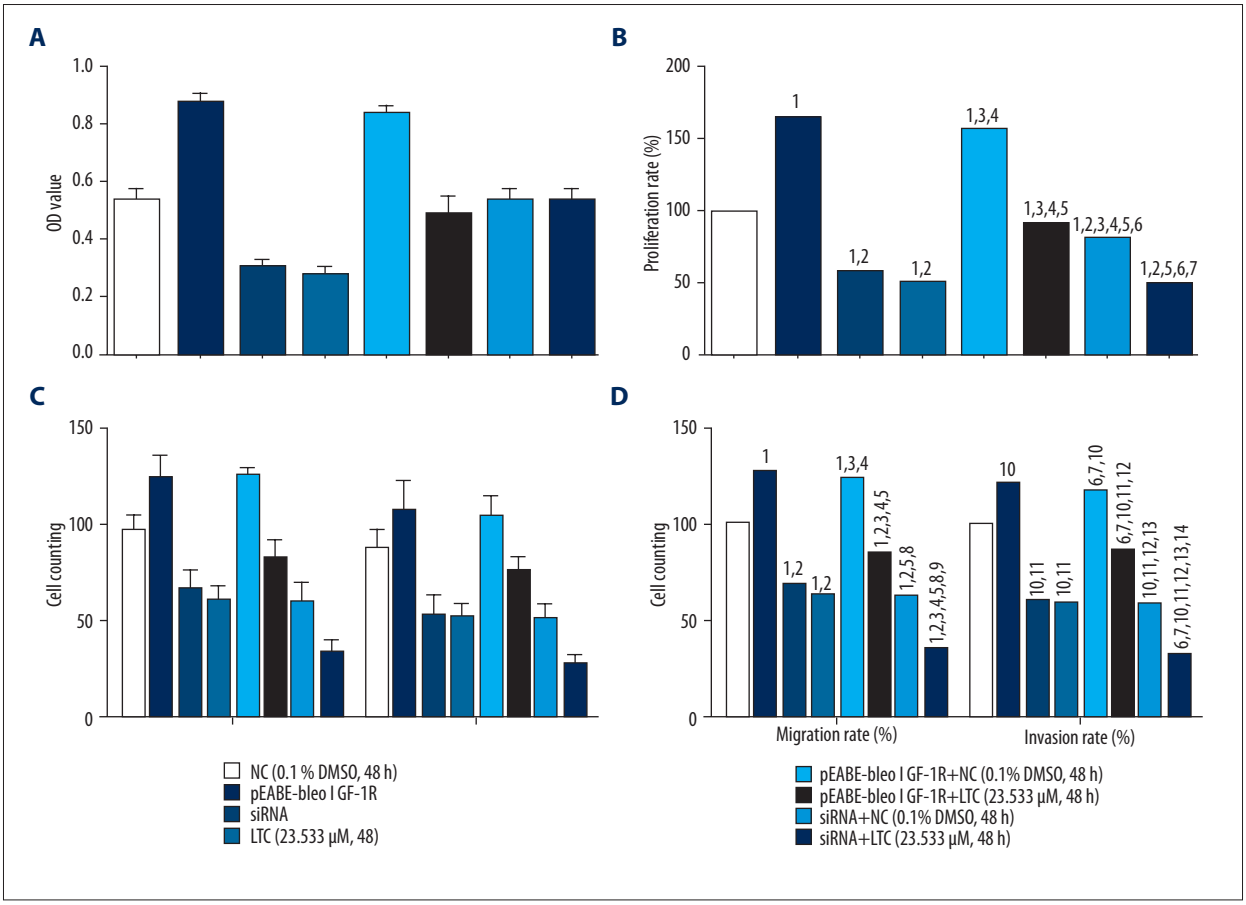
Table 3. Effect of LTC and/or siRNA targeting IGF-1R on MG63 cells proliferation (X±SDs).

	OD value	Proliferation rate (%)
NC (0.1% DMSO, 48 h)	0.534±0.041	100.00
pEABE-bleo IGF-1R	0.874±0.033	164.67[1]
siRNA	0.305±0.024	57.12[1,2]
LTC (23.533μM, 48h)	0.270±0.034	50.56[1,2]
pEABE-bleo IGF-1R+NC (0.1% DMSO, 48 h)	0.836±0.027	157.55[1,3,4]
pEABE-bleo IGF-1R +LTC (23.533 μM, 48 h)	0.491±0.058	91.95[2,3,4,5]
siRNA+NC (0.1% DMSO, 48 h)	0.434±0.025	81.27[1,2,3,4,5,6]
siRNA+LTC (23.533 μM, 48 h)	0.265±0.043	49.63[1,25,6,7]

Type-1 insulin-like growth factor receptor; LTC – Leptocarpin; NC – negative control group; DMSO – dimethylsulfoxide; [1] comparing to NC (0.1% DMSO, 72 h), $P<0.05$; [2] comparing to pEABE-bleo IGF-1R, $P<0.05$; [3] comparing to siRNA, $P<0.05$; [4] LTC (23.533 μM, 48 h), $P<0.05$; [5] comparing to pEABE-bleo IGF-1R+NC (0.1% DMSO, 48 h), $P<0.05$; [6] comparing to pEABE-bleo IGF-1R +LTC (23.533 μM, 48 h), $P<0.05$; [7] comparing to siRNA+NC (0.1% DMSO, 48 h), $P<0.05$.



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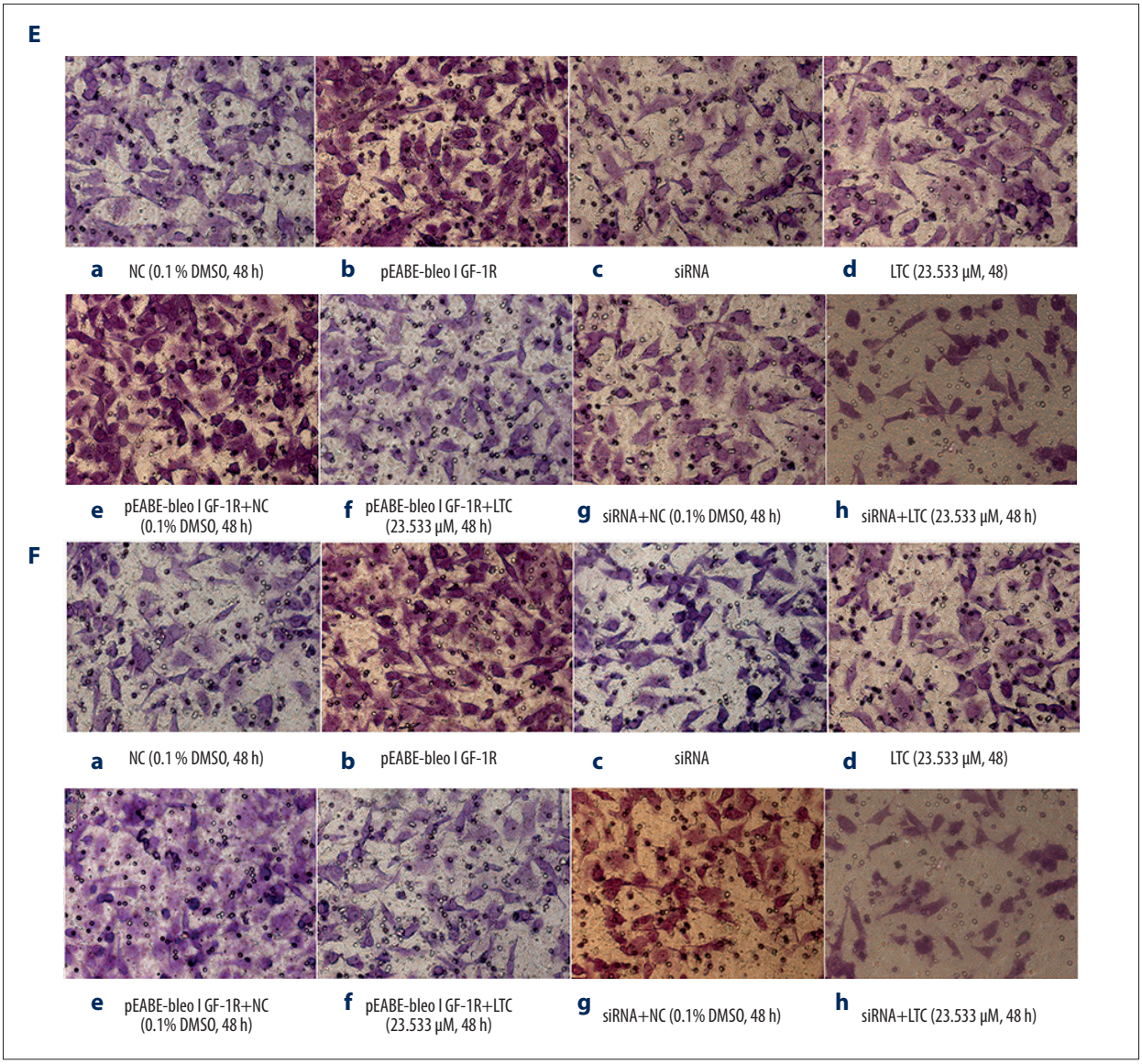

Figure 2. Effect of LTC on MG63 cell proliferation, migration, and invasion with different treatments. (**A**) Effect of LTC on MG63 cell proliferation. (**B**) Effect of LTC on MG63 cell proliferation rate ([1] compared to NC (0.1% DMSO, 72 h), $P<0.05$; [2] compared to pEABE-bleo IGF-1R, $P<0.05$; [3] compared to siRNA, $P<0.05$; [4] LTC (23.533 μM, 48 h), $P<0.05$; [5] compared to pEABE-bleo IGF-1R+NC (0.1% DMSO, 48 h), $P<0.05$;[6] compared to pEABE-bleo IGF-1R +LTC (23.533 μM, 48 h), $P<0.05$; [7] compared to siRNA+NC (0.1% DMSO, 48 h), $P<0.05$). (**C**) Effect of LTC on MG63 cell migration and invasion. (**D**) Effect of LTC on MG63 cell migration and invasion rates ([1] compared to NC (0.1% DMSO, 48 h) of migration, $P<0.05$; [2] compared to pEABE-bleo IGF-1R of migration, $P<0.05$; [3] compared to siRNA of migration, $P<0.05$; [4] compared to LTC (23.533 μM, 48 h) of migration, $P<0.05$; [5] compared to pEABE-bleo IGF-1R+ NC (0.1% DMSO, 48 h) of migration, $P<0.05$; [8] compared to pEABE-bleo IGF-1R+ LTC (23.533 μM, 48 h) of migration, $P<0.05$; [9] compared to siRNA+NC (0.1% DMSO, 48 h) of migration, $P<0.05$; [10] compared to NC (0.1% DMSO, 48 h) of invasion, $P<0.05$; [11] compared to pEABE-bleo IGF-1R of invasion, $P<0.05$; [6] compared to siRNA of invasion, $P<0.05$; [7] compared to LTC (23.533 μM, 48 h) of invasion, $P<0.05$; [12] compared to pEABE-bleo IGF-1R+ NC (0.1% DMSO, 48 h) of invasion, $P<0.05$; [13] compared to pEABE-bleo IGF-1R+ LTC (23.533 μM, 48 h) of invasion, $P<0.05$; [14] compared to siRNA+NC (0.1% DMSO, 48 h) of invasion, $P<0.05$). (**E**) Effect of LTC on MG63 cell migration. (**F**) Effect of LTC on MG63 cell invasion.

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Table 4. Effect of LTC and/or siRNA targeting IGF-1R on MG63 cells migration and invasion (X±SDs).

	Migration	Migration rate (%)	Invasion	Invasion rate (%)
NC (0.1% DMSO, 48 h)	96.17±8.44	100	87.56±9.10	100
pEABE-bleo IGF-1R	122.94±12.50	127.84[1]	106.38±15.12	121.49[10]
siRNA	66.33±10.05	68.97[1,2]	52.56±10.28	60.03[10,11]
LTC (23.533 μM, 48 h)	60.24±7.95	62.64[1,2]	51.89±7.03	59.26[10,11]
pEABE-bleo IGF-1R+ NC (0.1% DMSO, 48 h)	119.48±8.35	124.24[1,3,4]	103.16±10.75	117.82[6,7,11]
pEABE-bleo IGF-1R+ LTC (23.533 μM, 48 h)	82.15±9.84	85.42[1,2,3,4,5]	76.09±6.51	86.90[6,7,11,12]
siRNA+NC (0.1% DMSO, 48 h)	60.17±9.61	62.57[1,2,5,8]	51.29±7.52	58.58[10,11,12,13]
siRNA+LTC (23.533 μM, 48 h)	33.80±6.25	35.15[1,2,3,4,5,8,9]	28.06±4.73	32.05[6,7,10,11,12,13,14]

Type-1 insulin-like growth factor receptor; LTC – Leptocarpin; NC – negative control group; DMSO, dimethylsulfoxide; [1] comparing to NC (0.1% DMSO, 48 h) of migration, $P<0.05$; [2] comparing to pEABE-bleo IGF-1R of migration, $P<0.05$; [3] comparing to siRNA of migration, $P<0.05$; [4] comparing to LTC (23.533 μM, 48 h) of migration, $P<0.05$; [5] comparing to pEABE-bleo IGF-1R+ NC (0.1% DMSO, 48 h) of migration, $P<0.05$; [8] comparing to pEABE-bleo IGF-1R+ LTC (23.533 μM, 48 h) of migration, $P<0.05$; [9] comparing to siRNA+NC (0.1% DMSO, 48 h) of migration, $P<0.05$; [10] comparing to NC (0.1% DMSO, 48 h) of invasion, $P<0.05$; [11] comparing to pEABE-bleo IGF-1R of invasion, $P<0.05$; [6] comparing to siRNA of invasion, $P<0.05$; [7] comparing to LTC (23.533 μM, 48 h) of invasion, $P<0.05$; [12] comparing to pEABE-bleo IGF-1R+ NC (0.1% DMSO, 48 h) of invasion, $P<0.05$; [13] comparing to pEABE-bleo IGF-1R+ LTC (23.533 μM, 48 h) of invasion, $P<0.05$; [14] comparing to siRNA+NC (0.1% DMSO, 48 h) of invasion, $P<0.05$.

treatment for 72 h both obviously suppressed MG63 cell proliferation. These results indicate that a high concentration of LTC had obvious effects on MG63 cell proliferation, in agreement with previous reports. By analysis the IC50, we chose 23.533 μM LTC treatment for 48 h in the following experiments. By detecting migration and invasion, we found that 23.533 μM LTC treatment for 48 h obviously suppressed MG63 cell migration and invasion, supporting the previous investigations mentioned above.

IGF-1R, a tetrameric transmembrane receptor tyrosine kinase, is expressed widely in normal human tissues. Increasing evidence suggests that IGF-1R is associated with progression of human tumors [29]. Jasminka et al. [30] found that IGF-1R was overexpressed in endometrial carcinoma. According to Martinez et al. [14], LTC had no effect on DNA or RNA synthesis but produced marked effects on the protein biosynthesis in cancerous cell lines, which showed the biological activity of LTC, as an anti-neoplastic agent of natural origin.

To confirm the promotion of overexpressed IGF-1R on tumor cell proliferation, we referred to previous studies [16,17] and detected the IGF-1R expression in MG63 cells. We found that LTC treatment obviously inhibited IGF-1R expression in MG63 cells, similar to results of siRNA silencing IGF-1R. Even after overexpressing IGF-1R, LTC still decreased IGF-1R expression. By detecting proliferation, migration, and invasion, we found that MG63 cell proliferation, migration, and invasion were obviously suppressed by LTC treatment, even in the MG63 cells with overexpressed IGF-1R. Additionally, siRNA also suppressed MG63 cell proliferation, migration, and invasion, but the effect of siRNA combined with LTC was more powerful than LTC treatment alone. These results suggest that IGF-1R has important roles in LTC's suppression of MG63 cells proliferation, migration, and invasion.

Claudia et al. [31] also found that LTC decreased cell viability of cancer cell lines via inducing an apoptotic process and inhibiting the NF-κB signaling pathway. LTC is also an effective inhibitor of NF-κB, which is involved in cell growth, including apoptosis and proliferation, and even migration and invasion. Therefore, other targets that LTC acts on might be critical proteins in the NF-κB signaling pathway. Unfortunately, we did not detect the role of IGF-1R in the NF-κB signaling pathway in LTC treatment of MG63 cells. Animal experiments are needed to confirm this hypothesis *in vivo*.

Conclusions

We found that LTC suppressed osteosarcoma proliferation, migration, and invasion. IGF-1R is one of the targets in LTC's suppression of osteosarcoma, which provides clinical treatments for osteosarcoma with a new drug and molecular target. Further investigations are needed to demonstrate the role of IGF-1R in LTC's suppression of osteosarcoma, and animal experiments are also needed.

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Statement

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