OLR1 promotes cell proliferation and invasion while represses apoptosis via regulating histone H3 lysine 18 lactylation-mediated LEMD1 in papillary thyroid cancer

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Abstract. Oxidized low density lipoprotein receptor 1 (OLR1) regulates glycolytic metabolism, lactate production and carcinogenesis, but its role in papillary thyroid cancer (PTC) is still unclear. The present study aimed to investigate the effect of OLR1 modification on PTC cellular functions and its interaction with histone H3 lysine 18 lactylation (H3K18la) and downstream molecular mechanisms. Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1; OLR1 coding protein) and H3K18la expression was detected in twenty pairs of PTC and non-tumor tissues by immunohistochemistry. Overexpressed OLR1 and small interfering (si)RNA were transfected into two PTC cell lines (TPC-1 and IHH-4 cells). After which, oxamate (to reduce lactate production) or LEM domain containing 1 (LEMD1) siRNA combined with OLR1 overexpression plasmid was used to treat PTC cell lines. Furthermore, PTC xenograft mice were constructed for *in vivo* verification. LOX-1 and H3K18la expression was upregulated in PTC tissues versus non-tumor tissues and LOX-1 expression positively associated with H3K18la expression in PTC tissues. OLR1 overexpression facilitated cell proliferation and invasion but repressed cell apoptosis in PTC cell lines, while OLR1 siRNA revealed an opposite effect. OLR1 positively regulated lactate production, H3K18la expression and LEMD1 expression. Furthermore, a chromatin immunoprecipitation assay confirmed a direct binding between H3K18la and the LEMD1 promoter. H3K18la expression was lowered by oxamate-induced lactate reduction, which also attenuated the effect of OLR1 overexpression on PTC cell proliferation, apoptosis and invasion and downregulated LEMD1 expression. Moreover, LEMD1 siRNA weakened the effect of OLR1 overexpression on PTC cell proliferation, apoptosis and invasion. *In vivo* experiments revealed that OLR1 overexpression elevated tumor volume and weight and upregulated the expressions of LEMD1 and H3K18la; while LEMD1 knockdown reduced tumor volume and weight. In conclusion, OLR1 promotes PTC proliferation and invasion, while represses apoptosis through regulating H3K18la-mediated LEMD1.

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Introduction

Thyroid cancer is one of the most common malignancies that accounts for ~2.5% of all cancer cases (1). Based on the latest cancer statistics reports, the global number of new cases and mortality from thyroid cancer per year reached 821,173 and 47,485, respectively, among which China makes up 466,118 of new cases and 11,564 of mortalities (2,3). Thyroid cancer is histologically classified into three main subtypes: i) Differentiated [primarily comprising papillary thyroid cancer (PTC) and follicular thyroid cancer]; ii) poorly differentiated; and iii) undifferentiated (anaplastic) thyroid cancer (4); of these, PTC is the most prevalent accounting for 80-90% of thyroid cancer cases (5). After standard treatment involving radical resection followed by adjuvant endocrine therapy or radioactive iodine ablation, the majority of patients with PTC are able to obtain long-term survival. However, for some patients, particularly those with metastasis or high recurrent risk, the prognosis is still unsatisfactory (6,7). Thus, deep investigation into the pathogenesis underlying PTC initiation and progress remains important to further improve the outcomes.

Histone lactylation is a recently discovered post-translational modification (PTM) of lysine (K) residues, characterized by the covalent attachment of lactate to the ε-amino group (-NH□) of histones via an ester bond, forming lactylated lysine (8,9). Although the concept of histone lactylation was recently proposed, its relation to carcinogenesis has attracted attention. Several recent reviews have preliminarily summarized its implications in the pathogenesis of cancers via multiples mechanisms, including metabolic reconstruction, immune infiltration, angiogenesis and tumor invasiveness (10-12). Among the histone lactylated sites, histone H3 lysine 18 lactylation (H3K18la) is identified in numerous types of tissues and cells (13) and is the most extensively studied site for histone lactylation (14). Moreover, the function of H3K18la is largely investigated in a number of complex diseases such as cancer, respiratory

disease, renal disease and neurodegenerative disease by recent studies (15-18). Particularly in the cancer field, H3K18la is able to promote the progression of gastric, lung, breast and ovarian cancer (15,19-21). However, the role of H3K18la in PTC pathogenesis is still not reported. Considering the general functions of histone lactylation and the specific effects of H3K18la in cancers (10-12,15,19-21), it is hypothesized that H3K18la is likely involved in the pathogenesis of PTC.

Oxidized low density lipoprotein receptor 1 (OLR1), the gene encoding lectin-like oxidized low density lipoprotein receptor-1 (LOX-1), belongs to the C-type lectin superfamily and is a 50 kDa transmembrane glycoprotein that recognizes various ligands including oxidized low-density lipoprotein (oxLDL) and participates in various metabolic activities (22-24). Notably, OLR1 is able to facilitate the progression of cancers through multiple mechanisms such as regulating proliferation, epithelial-mesenchymal transition (EMT), angiogenesis, immune microenvironment and drug resistance (23,25). For example, one study indicated that OLR1 promoted the migration, invasion and EMT of gastric cancer through the PI3K/AKT/GSK3β pathway (26). Another study reported that the activation of OLR1 by oxLDL elevated the expressions of MMP2, MMP9 and VEGF, thereby inducing tumor angiogenesis and growth in prostate cancer (27). Moreover, OLR1 knockdown represses colon cancer growth and chemoresistance by inhibiting glycolytic metabolism and lactate production (28). However, the relation between OLR1 and PTC is rarely investigated. Therefore, in the present study, a preliminary investigation was performed and it was found that OLR1 was upregulated in thyroid cancer tissues versus non-tumor tissues and worse overall survival (OS) was predicted in patients with thyroid cancer via the Gene Expression Profiling Interactive Analysis (GEPIA) database. Moreover, it was also observed that LOX-1 (OLR1 coding protein) and H3K18la were upregulated in PTC tissues compared with non-tumor tissues and LOX-1 was positively associated with H3K18la in PTC tissues. Based on the prior studies and our preliminary investigation, a possible connection between OLR1 and H3K18la in PTC was hypothesized and they might be closely implicated in the PTC pathogenesis.

Based on the aforementioned information, the present study aimed to investigate the effect of OLR1 modification on PTC proliferation, apoptosis and invasion and its interaction with H3K18la and downstream molecular mechanisms.

Materials and methods

GEPIA public database analyses. The 'Expression Boxplot', 'Expression Stage Plot' and 'Survival Plots' modules on the GEPIA database (http://gepia.cancer-pku.cn/index.html) were used to analyze the expression of OLR1 in tumor and adjacent tissues and its correlation with tumor stage and survival in thyroid carcinoma.

Immunohistochemistry (IHC). A total of 20 PTC patients (between April 2024 and July 2025) were included, with median age of 46.5 years (range: 29-58 years). Among them 3 (15%) were males and 17 (85%) were females. Paired tumor and adjacent tumor tissues (~1 cm from the tumor margin) were obtained both retrospectively using stored samples and prospectively. The tissues were fixed in 4% paraformaldehyde for 24 h, embedded in paraffin and cut into 4-μm slices. The slides were deparaffinized, rehydrated and antigen-restored, successively. After removing the endogenous peroxidase in 3% H_2O_2, the slices were blocked by 5% goat serum (Wuhan Servicebio Technology Co., Ltd.) for 30 minutes (min) at room temperature and cultivated in anti-LOX-1 antibody (1:200; cat. no. DF6522, Affinity Biosciences) or anti-H3K18la (1:500; cat. no. PTM-1406RM, PTM BIO) at 4˚C overnight. Subsequently, the slices were incubated with anti-rabbit-HRP secondary antibody (1:2,000; cat. no. GB23303, Wuhan Servicebio Technology Co., Ltd.) at room temperature for 30 min. Finally, a DAB kit (Wuhan Servicebio Technology Co., Ltd.) was used to develop the color. The slices were counterstained by hematoxylin (Wuhan Servicebio Technology Co., Ltd.) at room temperature for 3 min. The images were captured under an inverted microscope (Motic Incorporation, Ltd.). The slices were analyzed by two pathologists, independently. The IHC score was the result of the staining intensity (score of 0, 1, 2 or 3) multiplied by the staining percentage (score of 1, 2, 3 or 4), of which the highest was 12. The final score was the average assessment by two pathologists. The present study was approved by the Ethics Committee of Harbin Medical University Cancer Hospital with approval number YD2024-13 on November 4, 2024. The Ethics Committee formally waived the requirement for additional informed consent for the study. Moreover, participants had provided written consent for the secondary use of biological specimens and medical data as part of routine clinical procedures at our institution.

Cell culture. The human PTC cell lines TPC-1 (Cellverse Bioscience Technology Co., Ltd.) and IHH-4 (Cellverse Bioscience Technology Co., Ltd.) were obtained. RPMI-1640 (Procell Life Science & Technology Co., Ltd.) with 10% fetal bovine serum (Procell Life Science & Technology Co., Ltd.) was used to culture the cells in 5% CO_2 and at 37˚C.

OLR1 plasmid and siRNA transfection. Negative control (NC) overexpression plasmid (oeNC; 0.8 μg; Shanghai GenePharma Co., Ltd.), OLR1 overexpression plasmid (oeOLR1; 0.8 μg; Shanghai GenePharma Co., Ltd.), NC siRNA (siNC; 50 nM; Shanghai GenePharma Co., Ltd.) and OLR1 siRNA (siOLR1; 50 nM; Shanghai GenePharma Co., Ltd.) were transfected into TPC-1 and IHH-4 cells at 37˚C for 6 h. Transfect-mate (Shanghai GenePharma Co., Ltd.) was applied to complete the transfection. At 48 h after transfection, LOX-1 (OLR1 coding protein) expression, LDHA expression, cell proliferation, cell apoptosis, cell invasion, lactate concentration, H3K18la expression and LEM domain containing 1 (LEMD1) expression were assessed. The siRNA sequences were: siOLR1 (sense, 5'-CAGCUGAUCUGGACUUCAUTT-3', antisense, 5'-AUGAAGUCCAGAUCAGCUGTT-3') and siNC (sense, 5'-UUCUCCGAACGUGUCACGUTT-3', antisense, 5'-ACGUGACACGUUCGGAGAATT-3').

Lactate and oxamate incubation. The TPC-1 and IHH-4 cells were incubated with 10 mM L-sodium lactate (Shanghai Aladdin Biochemical Technology Co., Ltd.) and 10 mM sodium oxamate (Shanghai Aladdin Biochemical Technology

Co., Ltd.), respectively. H3K18la expression and H3K18la enrich level on the LEMD1 promoter were evaluated.

Oxamate incubation and OLR1 plasmid transfection. oeNC and oeOLR1 were transfected into TPC-1 and IHH-4 cells. Meanwhile, 10 mM sodium oxamate was added and incubated with the cells. Afterwards, H3K18la expression, LEMD1 expression, cell proliferation, cell apoptosis and cell invasion were measured.

LEMD1 siRNA and OLR1 plasmid transfection. oeNC (0.8 μg) + siNC (50 nM), oeOLR1 (0.8 μg) + siNC (50 nM), oeNC (0.8 μg) + LEMD1 siRNA (siLEMD1; 50 nM; Shanghai GenePharma Co., Ltd.) and oeOLR1 + siLEMD1 were transfected into TPC-1 and IHH-4 cells in the presence of Transfect-mate (Shanghai GenePharma Co., Ltd.) at 37˚C for 6 h. LOX-1 (OLR1 coding protein) expression, LEMD1 expression, cell proliferation, cell apoptosis and cell invasion were analyzed at 48 h after transfection. The siRNA sequences of siLEMD1 were listed: siLEMD1 (sense, 5'-GUGUCUGAGUGACUGUAAATT-3', antisense, 5'-UUUACAGUCACUCAGACACTT-3').

Cell proliferation, cell apoptosis and lactate assessment. Cell counting kit-8 reagent (Wuhan Servicebio Technology Co., Ltd.) was incubated with the cells at 0, 24, 48 and 72 h after transfection for 2 h. The OD value was read at 450 nm and a cell apoptosis kit (Wuhan Servicebio Technology Co., Ltd.) was used to analyze cell apoptosis 48 h after treatment. The cells were incubated in Annexin V and propidium iodide working solution for 15 min in darkness at room temperature. A BD FACSCalibur flow cytometer (BD Biosciences) was used to analyze the cells. The data was analyzed by FlowJo X (BD Biosciences). The percentage of early + late apoptotic cells was calculated. The concentration of lactate was analyzed by a Lactic Acid Content Assay Kit (Abbkine Scientific Co., Ltd.) according to the manufacturer's instructions 48 h after transfection.

Cell invasion. After transfection for 48 h, the cells were harvested. Transwell inserts (0.8 μm; Wuhan NEST Biotechnology Co., Ltd.) were cultured with Matrigel Matrix (BD Biosciences) at 37˚C for 1 h. After which, the cells ($2x10^4$) in phosphate buffer saline were loaded into the upper chamber of Transwell inserts and the lower chamber was filled with 10% FBS-containing RPMI-1640. The inserts were incubated for 24 h at 37˚C. Afterwards, the cells were stained in crystal violet solution (Wuhan Servicebio Technology Co., Ltd.) at room temperature for 5 min after being incubated with 4% paraformaldehyde at room temperature for 15 min. Finally, the invasive cells were counted with a microscope (Motic Incorporation, Ltd.).

Western blotting. The cells were lysed to extract total protein using RIPA buffer (Wuhan Servicebio Technology Co., Ltd.) 48 h after transfection or lactate/oxamate incubation. Total protein was quantified using a BCA kit (Wuhan Servicebio Technology Co., Ltd.). A total of 10 μg thermally denatured protein was loaded on 4-20% pre-cast gels, separated and transferred to a nitrocellulose membrane (Whatman plc; Cytiva). The membrane was then blocked in 5% skimmed milk (Wuhan Servicebio Technology Co., Ltd.) at 37˚C for 60 min. Primary antibodies, including anti-LOX-1 antibody (1:2,000; cat. no. DF6522, Affinity Biosciences), anti-LEMD1 antibody (1:1,000; cat.no.CSB-PA715035LA01HU, Cusabio Technology, LLC) anti-H3K18la (1:2,000; cat. no. PTM-1406RM, PTM BIO), anti-H3 antibody (1:5,000; cat. no. AF0863, Affinity Biosciences), LDHA antibody (1:2,000; cat. no. DF6280, Affinity Biosciences) and anti-GAPDH antibody (1:3,000; cat. no. GB15004, Wuhan Servicebio Technology Co., Ltd.), were cultivated with the membrane overnight at 4˚C. After which, anti-rabbit-HRP secondary antibody (1:20,000; cat. no. GB23303, Wuhan Servicebio Technology Co., Ltd.) was incubated with the membrane for 90 min. Finally, the protein bands were illuminated with an ECL kit (MeilunBio). The western blotting images were quantified using ImageJ software (v1.8; National Institutes of Health).

Chromatin immunoprecipitation (ChIP) assay. The cells ($1x10^7$) were crosslinked with formaldehyde for 10 min at 37˚C and suspended in sodium dodecyl sulfate buffer. After which, the cells were ultrasonically lysed (25% frequency; 30 sec on, 30 sec off, 20 cycles) on ice and centrifuged. The lysate (100 μl) was incubated with IgG (1:50) or H3K18la (1:50) antibody at 4˚C overnight, followed by incubation with protein A+G Agarose (Beyotime Biotechnology) for 1 h, and the 1 μl lysate was used as input. After incubation, the lysate was centrifuged (1,000 x g) at 4˚C for 10 min and washed with specific buffer (IP Elution Buffer), successively. After which, the precipitate was reverse crosslinked and the DNA was analyzed with Taq DNA Polymerase containing-SYBR Green qPCR Master Mix (Wuhan Servicebio Technology Co., Ltd.). The thermal cycle procedures were as follows: 95˚C for 2 min, 1 cycle; 95˚C for 15 sec, 61˚C for 30 sec, for 40 cycles. The data was calculated using the %Input method.

Xenograft experiment. The GV492 plasmid (Shanghai Genechem Co., Ltd.) and GV872 plasmid (Shanghai Genechem Co., Ltd.) were used to establish OLR1 overexpression plasmid and LEMD1 knockdown plasmid, respectively. The lentivirus was constructed through second generation system. The 20 μg OLR1 overexpression or LEMD1 knockdown plasmid, 15 μg pHelper 1.0 plasmid and 10 μg pHelper 2.0 plasmid were transfected into 293T cells (Cellverse Bioscience Technology Co., Ltd) at 37˚C for 6 h. The supernatant was collected to harvest lentivirus at 48 h after transfection. The TPC-1 cells were infected with OLR1 overexpression lentivirus, LEMD1 knock-down lentivirus alone or in combination (Shanghai Genechem Co., Ltd.). The multiplicity of infection (MOI) was 20. Moreover, control lentivirus (Shanghai Genechem Co., Ltd.) was infected the TPC-1 cells. The stably infected TPC-1 cells were selected with 1.5 μg/ml puromycin for 7 days. BALB/c nude mice with 4-6 weeks old and 18-22 g (Shanghai SLAC Laboratory Animal Co, Ltd.) were housed in specific pathogen-free facility and randomly assigned into four groups (three mice per group). The stably infected TPC-1 cells were inoculated on the right flank of the mice ($4x10^6$ cells per mouse). A caliper was used to measure tumor volume every week. The mice were sacrificed by cervical dislocation at four weeks. The tumors were harvested, weighted, fixed and cut

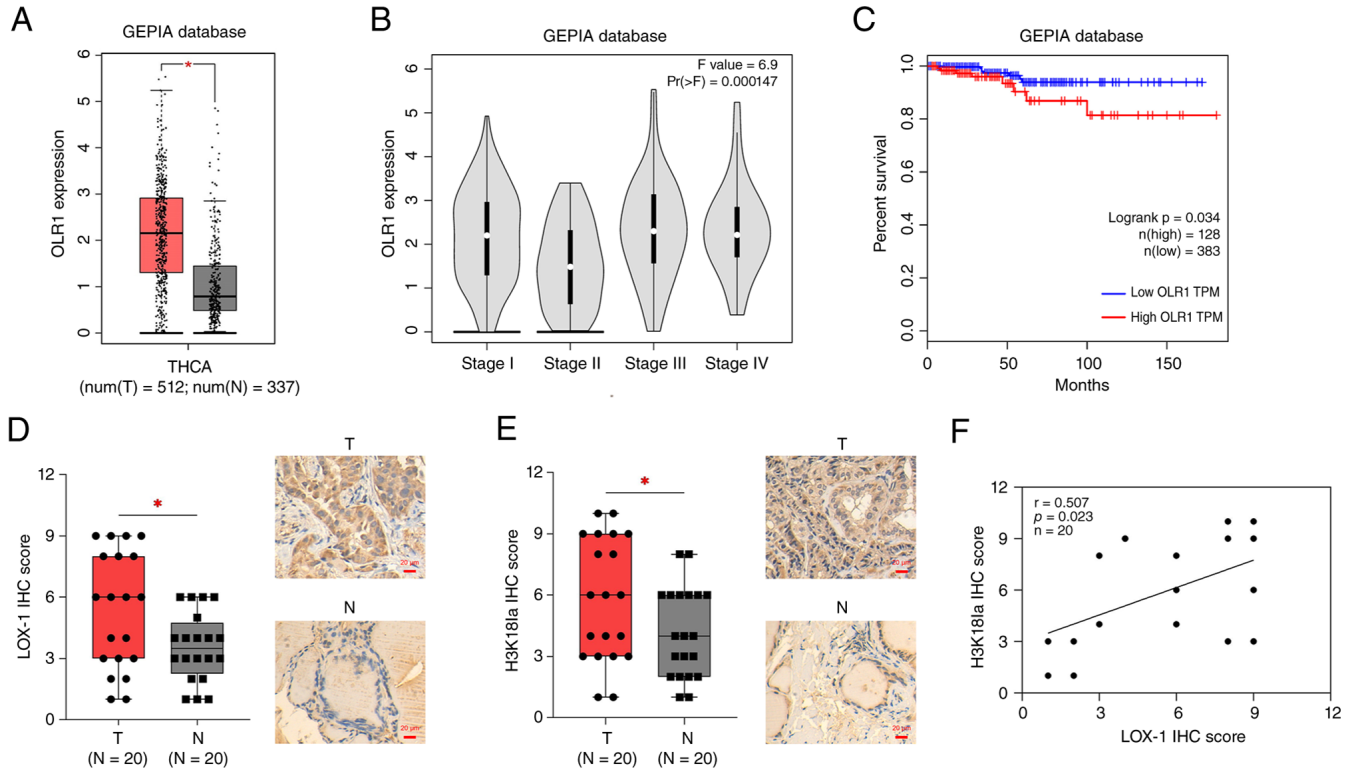

Figure 1. Expression of OLR1 and H3K18la in clinical samples. (A) OLR1 expression between thyroid cancer tissues and non-tumor tissues, (B) its correlation with TNM stage and (C) its correlation with OS in patients with thyroid cancer, via the GEPIA database. Expressions and IHC image examples of (D) LOX-1 and (E) H3K18la between PTC tissues and adjacent non-tumor tissues and (F) the correlation between OLR1 and H3K18la expression in PTC tissues. Magnification, x200. *P<0.05. OLR1, oxidized low density lipoprotein receptor 1; H3K18la, histone H3 lysine 18 lactylation; OS, overall survival; GEPIA, Gene Expression Profiling Interactive Analysis; IHC, immunohistochemistry; LOX-1, lectin-like oxidized low density lipoprotein receptor-1; PTC, papillary thyroid cancer.

into 4 μm sections. The IHC was performed as aforementioned, with the application of anti-LOX-1 antibody (1:200), anti-H3K18la antibody (1:500) and anti-LEMD1 antibody (1:100). The animal experiments were approved by the Ethics Committee of Harbin Medical University Cancer Hospital with approval number YD2024-13 and Wuhan Servicebio Institutional Animal Care and Use Committee (IACUC) with approval number 2025079-2.

Statistical analysis. All data in the present study were analyzed by GraphPad 9.0 software (Dotmatics). The experiments were performed in triplicate (n=3 in each group). Prior to conducting parametric tests, the normality and homogeneity of variance were analyzed by Shapiro-Wilk test and Brown-Forsythe test, respectively. If these assumptions were met, one-way analysis of variance with Tukey's or Dunnett's test was used for comparison among groups. A paired t-test was applied for comparison between tumor and paired-adjacent tumor groups. Pearson correlation coefficient test was adopted to analyze the correlation of LOX-1 and H3K18la expression. P<0.05 was considered to indicate a statistically significant difference.

Results

OLR1 and H3K18la expression in PTC. Referring to the analyses based on the GEPIA public database, OLR1 expression was upregulated in thyroid cancer tissues compared with non-tumor tissues (Fig. 1A) and its expression differed among patients with thyroid cancer at different TNM stages (Fig. 1B). Moreover, GEPIA also revealed that patients with thyroid cancer with high OLR1 expression presented a worse OS compared with those with low OLR1 expression (Fig. 1C). LOX-1 expression (OLR1 coding protein) was detected by IHC assay in 20 pairs of PTC tumor and adjacent non-tumor tissues for verification, which verified that LOX-1 expression was higher in PTC tumor tissues compared with adjacent non-tumor tissues (Fig. 1D). H3K18la expression was also detected by IHC assay and it showed a higher expression in PTC tumor tissues compared with adjacent non-tumor tissues (Fig. 1E). Moreover, LOX-1 expression was positively associated with H3K18la expression in PTC tumor tissues (Fig. 1F). LOX-1 expression was also positively associated with tumor size of PTC (Fig. S1).

OLR1 enhances PTC cell proliferation and invasion, but repressed cell apoptosis. LOX-1 expression was upregulated after oeOLR1 transfection, but downregulated after siOLR1 transfection in TPC-1 and IHH-4 cells (Fig. 2A and B), indicating the success of transfection. After which, cell proliferation, apoptosis and invasion were detected after transfection. oeOLR1 promoted cell proliferation, while siOLR1 inhibited cell proliferation in TPC-1 and IHH-4 cells (Fig. 2C). By contrast, oeOLR1 repressed the cell apoptosis rate but siOLR1 enhanced the cell apoptosis rate in TPC-1 and IHH-4 cells (Fig. 2D and E). Moreover, oeOLR1 increased the invasive cell count while siOLR1 reduced the invasive cell count in TPC-1 and IHH-4 cells (Fig. 2F and G).

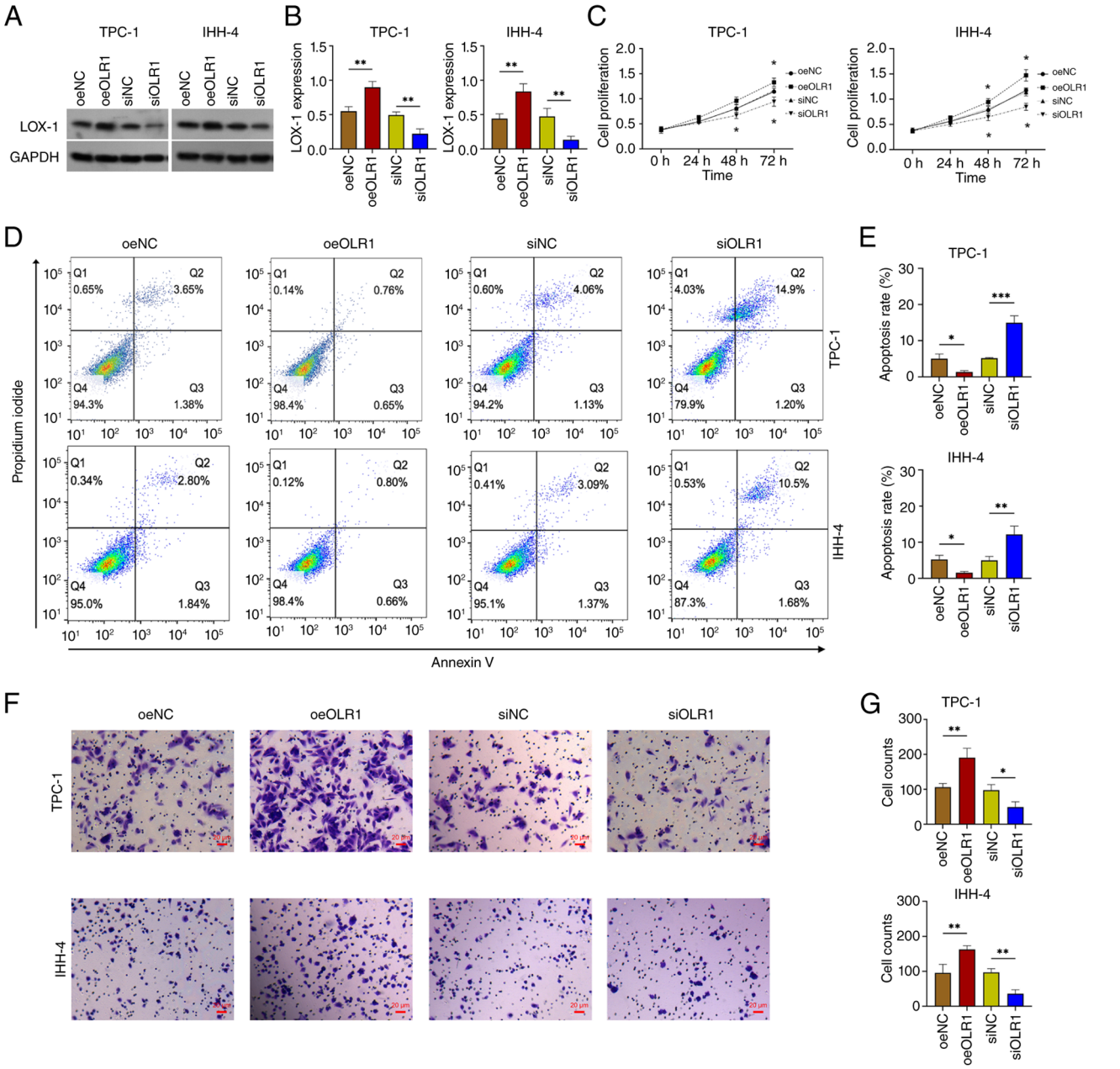

Figure 2. Effect of OLR1 modification on PTC proliferation, apoptosis and invasion. LOX-1 (A) western blotting image example and (B) its expression among oeNC, oeOLR1, siNC and siOLR1 groups. (C) Cell proliferation, (D) Annexin V/propidium iodide image example, (E) cell apoptosis rate, (F) Transwell image example and (G) invasive cell count among oeNC, oeOLR1, siNC and siOLR1 groups. Magnification, x200. *P<0.05; **P<0.01; ***P<0.001. OLR1, oxidized low density lipoprotein receptor 1; PTC, papillary thyroid cancer; LOX-1, lectin-like oxidized low density lipoprotein receptor-1; NC, negative control; oe, overexpression; si, siRNA.

OLR1 promotes H3K18la-mediated LEMD1 in PTC cells. oeOLR1 accelerated the production of lactate in TPC-1 and IHH-4 cells, but siOLR1 weakened it (Fig. S2). oeOLR1 also upregulated while siOLR1 downregulated LDHA expression in TPC-1 and IHH-4 cells (Fig. S3A and B). Moreover, oeOLR1 upregulated H3K18la expression, while siOLR1 downregulated H3K18la expression in TPC-1 and IHH-4 cells (Fig. 3A and B). Notably, a previous study reported that H3K18la is directly bound to the promoter of LEMD1 (29). Another study confirmed the oncogene role of LEMD1 in PTC (30). Therefore, the expression of LEMD1 and its relation to H3K18la was subsequently explored in the present study. oeOLR1 increased LEMD1 expression but siOLR1 decreased LEMD1 expression in TPC-1 and IHH-4 cells (Fig. 3A and C). In addition, oxamate reduced H3K18la expression, while lactate elevated H3K18la expression in TPC-1 and IHH-4 cells (Fig. 3D and E). Finally, the ChIP assay verified that H3K18la is bound to the LEMD1 promoter in TPC-1 and IHH-4 cells (Fig. 3F).

Lactate-mediated H3K18la may be implicated in the function of OLR1 in PTC cells. Oxamate was used to reduce lactate production along with plasmid transfection. Oxamate lowered H3K18la expression and attenuated the effect of oeOLR1 on H3K18la expression in TPC-1 and IHH-4 cells (Fig. 4A and B). In addition, oxamate downregulated LEMD1 expression and

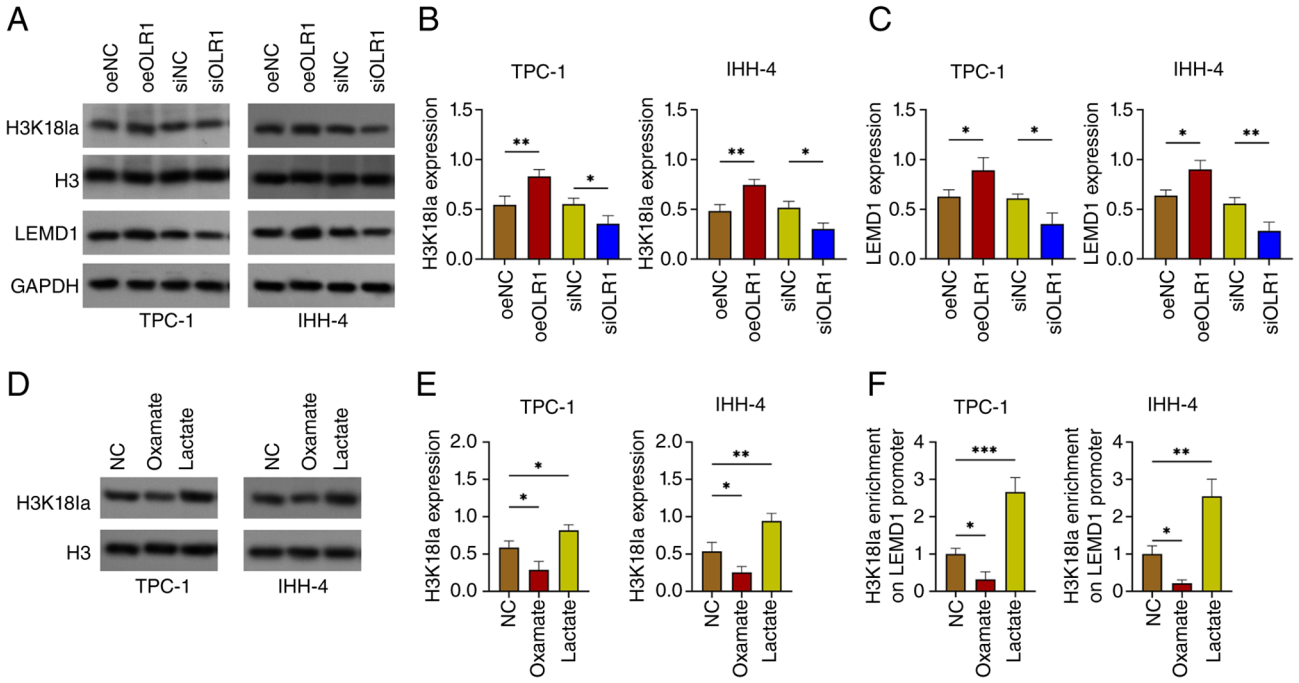

Figure 3. Effect of OLR1 modification on H3K18la and LEMD1. (A) Western blotting image example, (B) H3K18la expression and (C) LEMD1 expression among oeNC, oeOLR1, siNC and siOLR1 groups. (D) Western blotting image example and (E) H3K18la expression among NC, oxamate and lactate groups. (F) ChIP assay evaluating H3K18la enrichment on the LEMD1 promoter. *P<0.05; **P<0.01; ***P<0.001. OLR1, oxidized low density lipoprotein receptor 1; NC, negative control; oe, overexpression; si, siRNA; H3K18la, histone H3 lysine 18 lactylation; LEMD1, LEM domain containing 1.

weakened the effect of oeOLR1 on LEMD1 expression in TPC-1 and IHH-4 cells (Fig. 4A and C). Afterwards, oxamate decreased cell proliferation and increased the cell apoptosis rate in TPC-1 and IHH-4 cells, which also attenuated the effect of oeOLR1 on cell proliferation and apoptosis (Fig. 4D-F). Moreover, oxamate reduced the invasive cell count and attenuated the effect of oeOLR1 on the invasive cell count in TPC-1 and IHH-4 cells (Fig. 5A and B).

siLEMD1 attenuates the function of OLR1 in PTC cells. LEMD1 expression was reduced and the effect of oeOLR1 on LEMD1 expression was weakened by siLEMD1 transfection in TPC-1 and IHH-4 cells. However, LOX-1 expression was not affected by siLEMD1 transfection (Fig. 6A-C). siLEMD1 decreased cell proliferation but increased the cell apoptosis rate in TPC-1 and IHH-4 cells, which attenuated the effect of oeOLR1 on these functions (Fig. 6D-F). Furthermore, siLEMD1 repressed the invasive cell count and weakened the effect of oeOLR1 on the invasive cell count in TPC-1 and IHH-4 cells (Fig. 7A and B).

OLR1 promotes PTC tumor growth via LEMD1 in xenograft mice. oeOLR1 elevated tumor volume during observational period, maximum tumor volume and maximum tumor diameter (Fig. 8A and B). Moreover, oeOLR1 increased tumor weight (Fig. 8C) and upregulated the expressions of LEMD1 and H3K18la (Fig. 8D). By contrast, shLEMD1 reduced tumor volume during observational period, maximum tumor volume, maximum tumor diameter and weight, which also attenuated the effect of oeOLR1 in xenograft mice, but it did not affect the expressions of LOX-1 (OLR1 coding protein) and H3K18la. Moreover, the LOX-1 expression was upregulated in oeOLR1 group compared to Control group, indicating oeOLR1 transfection success; the LEMD1 expression was downregulated in shLEMD1 group compared with Control group, indicating shLEMD1 transfection success (Fig. 8D). Regarding the detailed values of maximum tumor volume and diameter: the maximum tumor volumes were 561±117 mm^3, 1136±171 mm^3, 241±18 mm^3, 674±45 mm^3 in the Control, oeOLR1, shLEMD1 and oeOLR1+shLEMD1 groups, respectively; and the maximum tumor diameters were 13.2±0.3 mm, 16.2±1.1 mm, 9.2±0.9 mm and 13.5±1.1 mm in the Control, oeOLR1, shLEMD1 and oeOLR1+shLEMD1 groups, respectively.

Discussion

OLR1 is aberrantly expressed and presents with prognostic value in several cancers (31-33). For example, OLR1 is largely overexpressed in head and neck squamous cell carcinoma (HNSCC) tissues compared with non-tumor tissues and its high expression is associated with worse OS in patients with HNSCC (31). OLR1 is also upregulated in ovarian cancer tissues and cell lines compared with non-tumor control tissues and normal cell lines, whose high expression is associated with elevated risk of recurrence and mortality in patients with ovarian cancer (32). Moreover, an upregulation of OLR1 in tumor tissues and a correlation between OLR1 upregulation and survival prognosis were discovered in patients with breast cancer (33). However, the related information is lacking in PTC. The present study preliminarily employed the GEPIA public database and found that OLR1 was upregulated in tumor tissues and associated with shorter OS in patients with thyroid cancer. Subsequently, via the IHC assay, the present study further confirmed that LOX-1

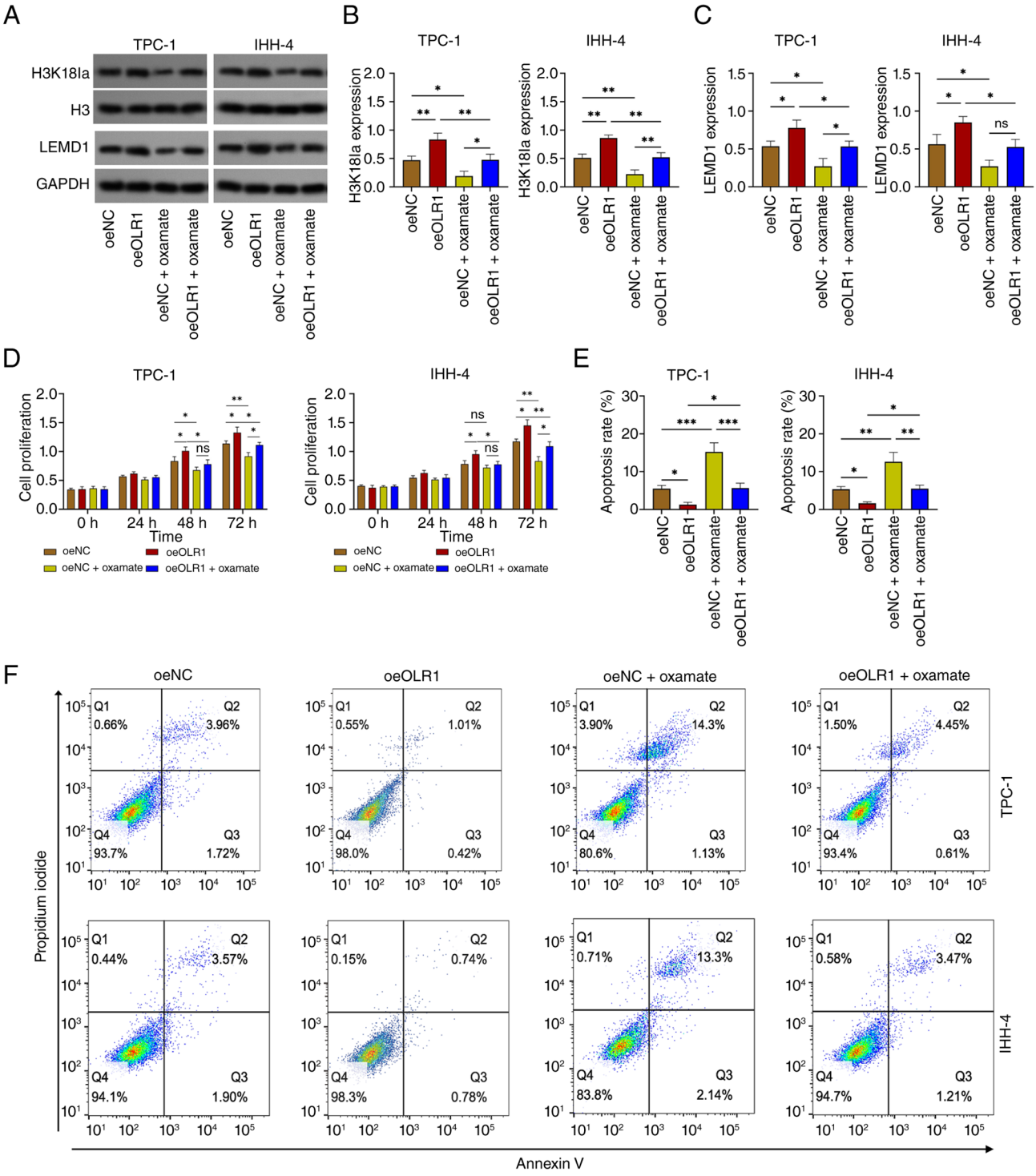

Figure 4. Effect of oxamate on H3K18la, LEMD1 and PTC proliferation and apoptosis. (A) Western blotting image example, (B) H3K18la expression and (C) LEMD1 expression among oeNC, oeOLR1, oeNC + oxamate and oeOLR1 + oxamate groups. (D) Cell proliferation, (E) cell apoptosis rate and (F) Annexin V/propidium iodide image example among oeNC, oeOLR1, oeNC + oxamate and oeOLR1 + oxamate groups. *P<0.05; **P<0.01; ***P<0.001. H3K18la, histone H3 lysine 18 lactylation; LEMD1, LEM domain containing 1; PTC, papillary thyroid cancer; oe, overexpression; NC, negative control; OLR1, oxidized low density lipoprotein receptor 1.

was upregulated in PTC tissues compared with adjacent non-tumor tissues. The explanation for this finding may be as follows: i) OLR1 serves as an oncogene via the regulation of tumor proliferation, EMT, angiogenesis, immune microenvironment and drug resistance (23,25) and thus, it is upregulated in PTC tumor tissues; and ii) referring to the function of OLR1 on regulating metabolic activities and their relation to carcinogenesis (22-24,28) and the finding that OLR1 promoted PTC proliferation and invasion, while repressed apoptosis in the subsequent experiments, OLR1 and its coding protein LOX-1 are considered to be upregulated in PTC tumor tissues. However, the present study did not assess the soluble level of LOX-1 in PTC patients, which could be explored in future studies.

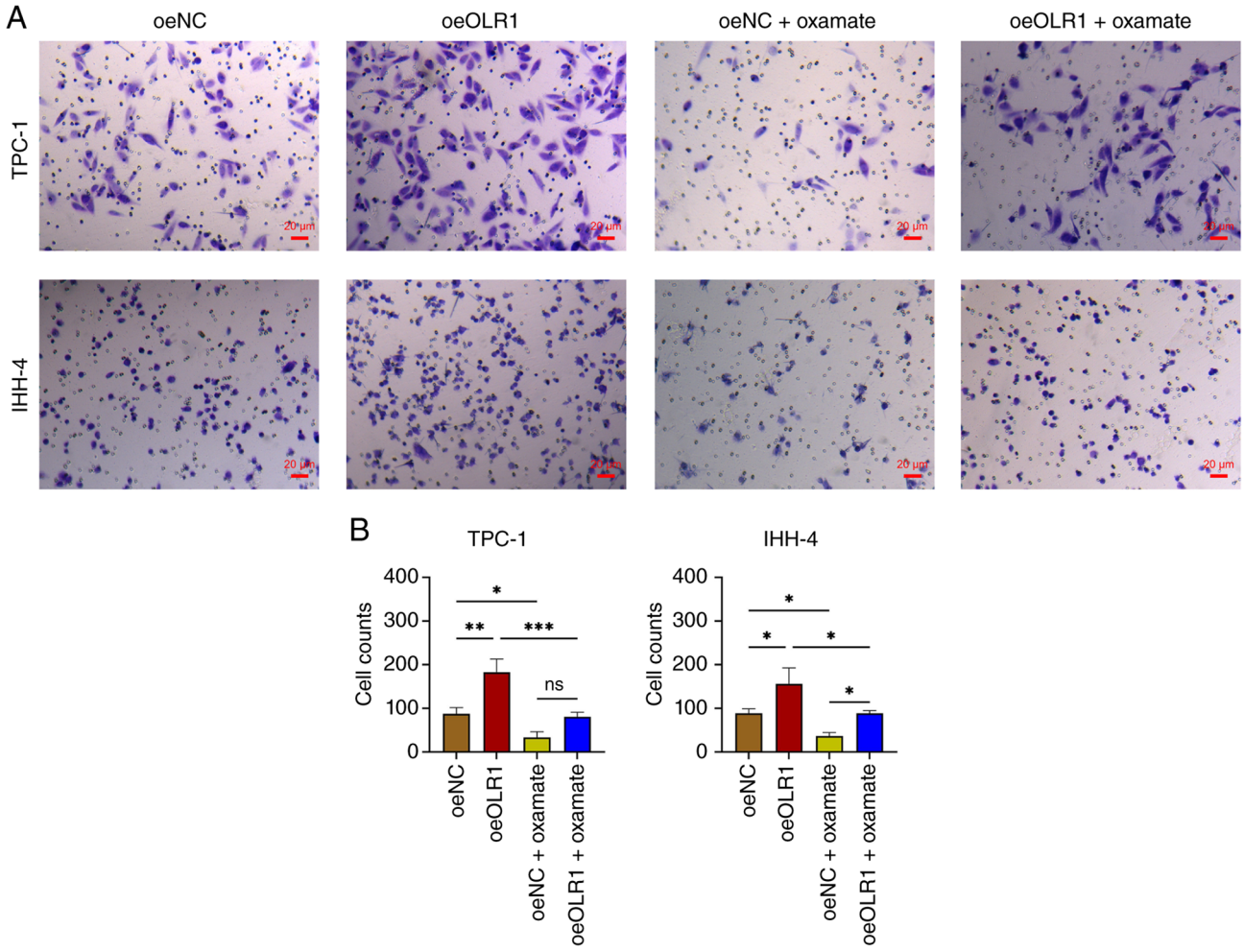

Figure 5. Effect of oxamate on PTC invasion. (A) Transwell image example and (B) invasive cell count among oeNC, oeOLR1, oeNC + oxamate and oeOLR1 + oxamate groups. Magnification, x200. *P<0.05; **P<0.01; ***P<0.001. PTC, papillary thyroid cancer; OLR1, oxidized low density lipoprotein receptor 1; oe, overexpression; NC, negative control.

In carcinogenesis, OLR1 promotes the progression of several types of cancer via multiple mechanisms (26-28,34-36). For example, OLR1 facilitates lung cancer growth, mobility and immune escape by activating the cancer-associated fibroblasts (34) and stimulates esophageal cancer tumorigenesis by regulating the RACK1-mediated MAP2K/MEK/MAPK/ERK pathway and autophagy (35). Furthermore, targeting OLR1 reduces oral cancer migration, invasion and stemness (36). However, data regarding OLR1 regulation during PTC pathogenesis are limited. Based on the preliminary findings of the present study that demonstrated that OLR1 was upregulated in tumor tissues and predicted a worse prognosis in patients with PTC, the present study further investigated the effect of OLR1 modification on PTC cellular functions. It found that OLR1 overexpression enhanced PTC proliferation and invasion but reduced PTC apoptosis and OLR1 knockdown revealed an opposite function. The explanation for this finding may be as follows: i) OLR1 positively regulates cancer glycolytic metabolism (28); ii) OLR1 controls several oncogene pathways such as PI3K/AKT, MAPK/MEK/ERK and Wnt/β-catenin (26,35,37); and iii) OLR1 activated H3K18la-mediated LEMD1 as discovered in subsequent experiments of the present study. Therefore, OLR1 is able to facilitate PTC proliferation and invasion but reduce its apoptosis.

The present study also found that OLR1 positively regulated H3K18la in PTC and lactate-mediated H3K18la possibly attenuated the effect of OLR1 on PTC proliferation, apoptosis and invasion. The explanations for this finding may be as follows: i) OLR1 regulates glycolytic metabolism and lactate production (28) and further upregulates H3K18la; therefore, OLR1 positively regulates H3K18la; ii) H3K18la promotes the progression of other cancers apart from PTC (15,19-21); therefore, it may facilitate PTC progression as that in other cancers; and iii) H3K18la may bind to LEMD1 and the latter is able to promote PTC progression (29,30); this is further verified in subsequent experiments.

LEMD1, also known as CT50 or LEMP-1, has been recognized as a newly identified member of the cancer-testis antigen family (38). Its expression pattern, primarily restricted to the testis under normal conditions but frequently reactivated in various tumor types, endows it with multiple attributes of an ideal biological target, holding significant promise for both cancer immunotherapy and diagnosis (38,39). Clinically, LEMD1 is upregulated

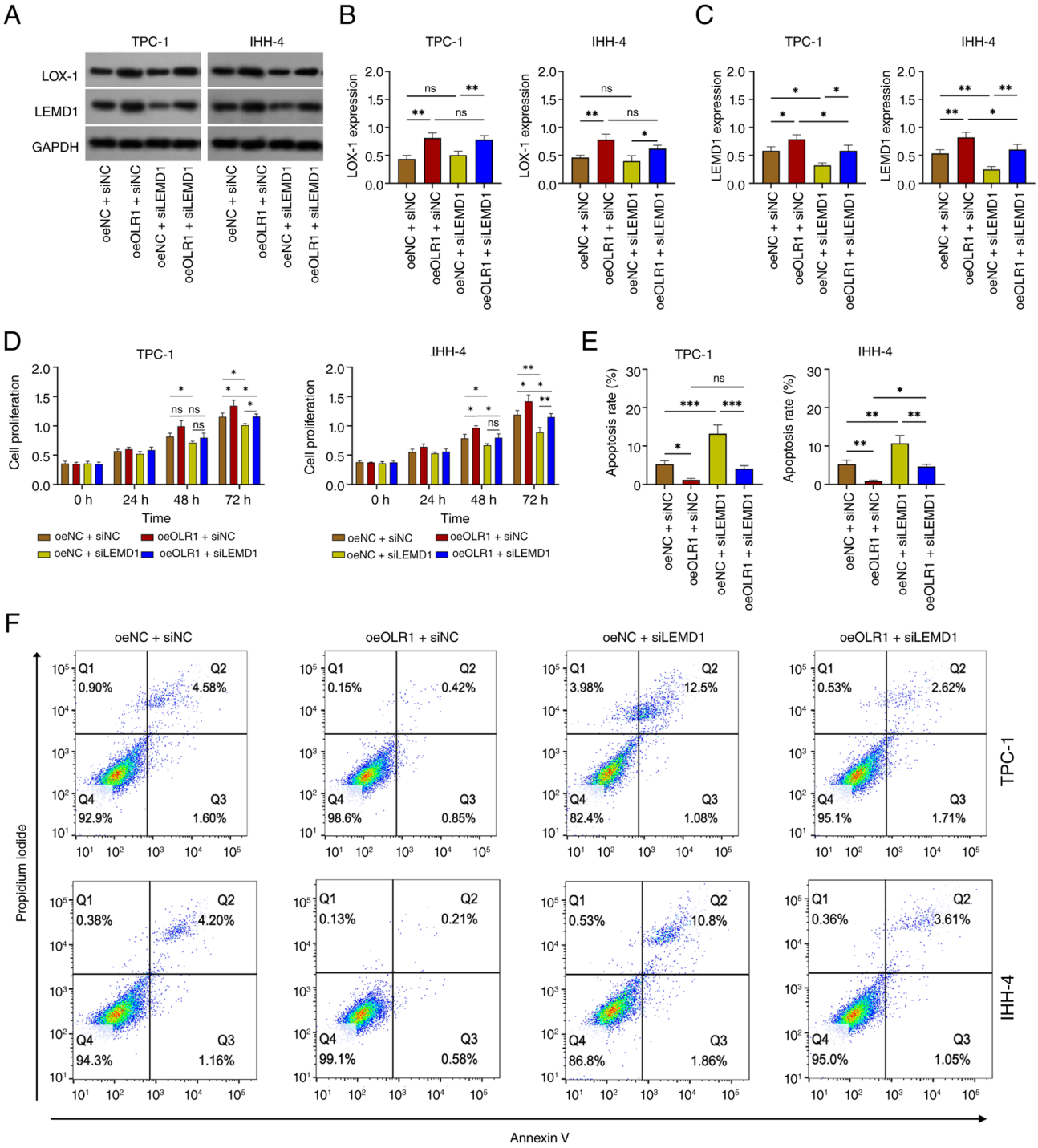

Figure 6. Effect of LEMD1 modification on PTC proliferation and apoptosis. (A) Western blotting image example, (B) LOX-1 and (C) LEMD1 expression among oeNC + siNC, oeOLR1 + siNC, oeNC + siLEMD1 and oeOLR1 + siLEMD1 groups. Cell proliferation (D), cell apoptosis rate (E) and Annexin V/propidium iodide image example (F) among oeNC + siNC, oeOLR1 + siNC, oeNC + siLEMD1 and oeOLR1 + siLEMD1 groups. *P<0.05; **P<0.01; ***P<0.001. LEMD1, LEM domain containing 1; PTC, papillary thyroid cancer; LOX-1, lectin-like oxidized low density lipoprotein receptor-1; OLR1, oxidized low density lipoprotein receptor 1; NC, negative control; oe, overexpression; si, siRNA.

in prostate cancer tissues compared to benign prostate hyperplasia tissues (40) and its associated with lymph node metastasis, advanced TNM stage and worse survival outcomes in patients with oral squamous cell carcinoma and non-small cell lung cancer (41,42). Moreover, LEMD1 is an independent factor predicting the recurrence risk of colorectal cancer (43). In the aspect of biological functions, LEMD1 has been previously reported to accelerate the growth, migration and invasion of pancreatic cancer via activating p53 and mTOR pathways (39). Moreover, LEMD1 knockdown suppresses proliferation, migration and colony formation, while restoring chemosensitivity via inactivating the ERK pathway in breast cancer (44). Particularly in PTC, LEMD1 promotes PTC proliferation, migration, invasion and EMT via the Wnt/β-catenin pathway (30). The present study found that LEMD1 knockdown repressed PTC

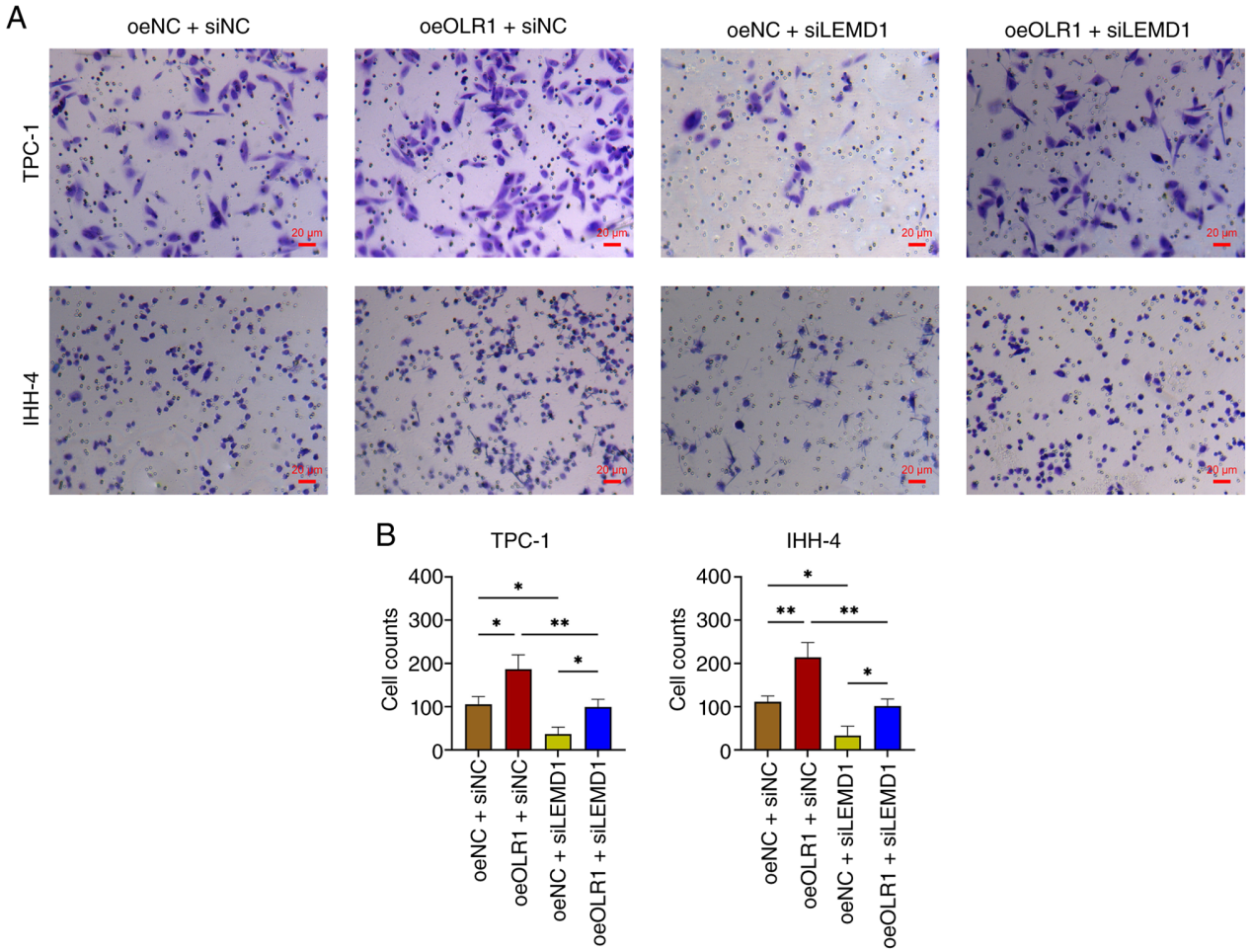

Figure 7. Effect of LEMD1 modification on PTC invasion. (A) Transwell image example and (B) invasive cell count among oeNC + siNC, oeOLR1 + siNC, oeNC + siLEMD1 and oeOLR1 + siLEMD1 groups. Magnification, x200. *P<0.05; **P<0.01. LEMD1, LEM domain containing 1; PTC, papillary thyroid cancer; OLR1, oxidized low density lipoprotein receptor 1; NC, negative control; oe, overexpression; si, siRNA.

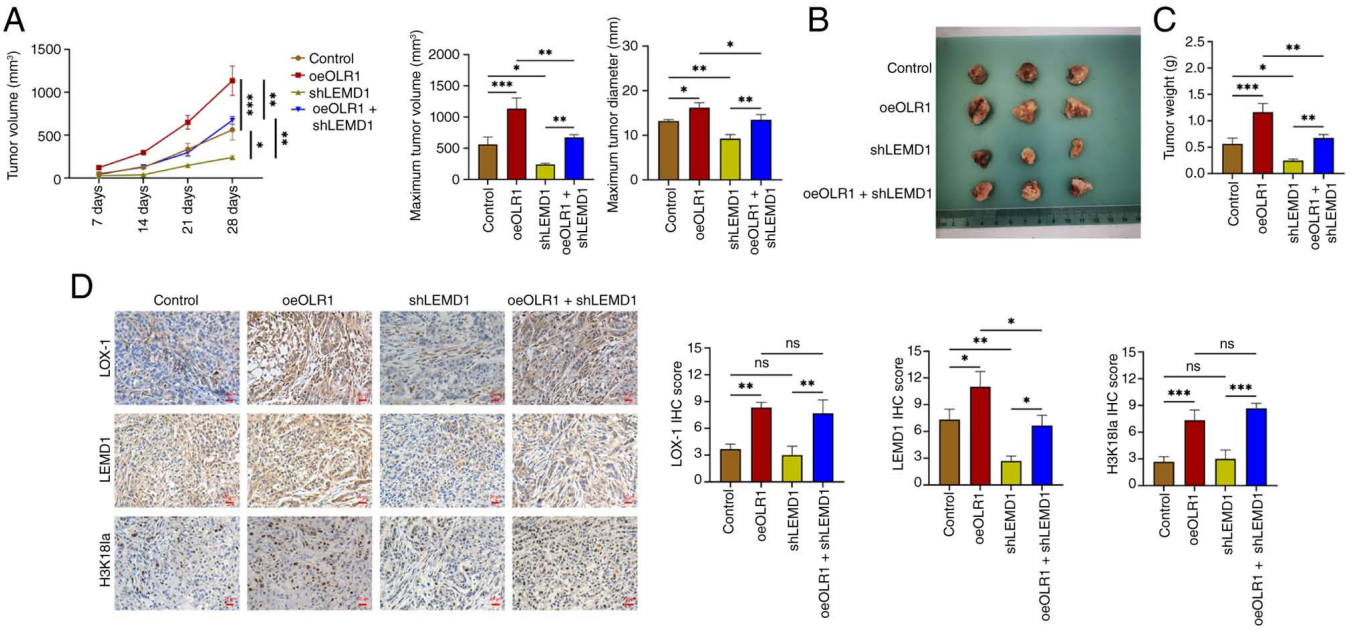

Figure 8. Effect of OLR1 and LEMD1 modification in PTC xenograft mice. (A) Tumor volume during observational period, maximum tumor volume and maximum tumor diameter, (B) tumor images, (C) tumor weight and (D) expressions of LOX-1, LEMD1 and H3K18la among Control, oeOLR1, shLEMD1 and oeOLR1 + shLEMD1 groups in PTC xenograft mice. Magnification, x200. *P<0.05; **P<0.01; ***P<0.001. OLR1, oxidized low density lipoprotein receptor 1; LEMD1, LEM domain containing 1; PTC, papillary thyroid cancer; LOX-1, lectin-like oxidized low density lipoprotein receptor-1; oe, overexpression; sh, short hairpin; H3K18la, histone H3 lysine 18 lactylation.

proliferation and invasion but enhanced its apoptosis, which attenuated the effect of OLR1 overexpression on these cellular functions in PTC. The explanation for this finding may be as follows: i) LEMD1 facilitates PTC progression via the Wnt/β-catenin pathway (30); and ii) LEMD1 regulates several other oncogene pathways such as p53, mTOR, ERK and PI3K/AKT (39,44,45).

The present study possessed some limitations. First, the BRAF V600E status of the PTC patients was not detected and the patients are still not recurrent at the time of the present study, therefore the correlation of OLR1/LOX-1 with BRAF V600E status and recurrent risk was not analyzed. Second, rescue experiments were not performed to validate the findings. Third, only the effect of OLR1 modification on LDHA expression was performed, while its effect on other glycolytic enzymes such as HK2 and PKM2 was not detected, which needs to be explored in the future studies.

In summary, OLR1 promoted proliferation and invasion while repressed apoptosis via regulating H3K18la-mediated LEMD1 in PTC. This may provide a novel insight regarding the involvement of OLR1 and histone lactylation in the pathogenesis of PTC. However, further validation is still required.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

JZ conceived and designed the study. JY collected the data. RP conducted the data analysis. BL and LC contributed to the interpretation of the data. LC drafted the manuscript, while all authors provided critical revisions. JZ and JY confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Harbin Medical University Cancer Hospital with approval number YD2024-13 on November 4, 2024. The Ethics Committee formally waived the requirement for additional informed consent for this study. Moreover, participants had provided written consent for the secondary use of biological specimens and medical data as part of routine clinical procedures at the Harbin Medical University Cancer Hospital. The animal experiments were approved by the Ethics Committee of Harbin Medical University Cancer Hospital with approval number YD2024-13 and Wuhan Servicebio Institutional Animal Care and Use Committee with approval number 2025079-2.

Patient consent for publication

The Ethics Committee formally waived the requirement for additional informed consent for this study. Moreover, participants had provided written consent for the secondary use of biological specimens and medical data as part of routine clinical procedures at our institution.

Competing interests

The authors declare that they have no competing interests.

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