Article

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Lactate-Induced H3K18 Lactylation Attenuates T-Cell Activation and Upregulates PD-L1 in Esophageal Squamous Cell Carcinoma

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Background: Immune checkpoint blockade targeting the Programmed Death-1 (PD-1)/Programmed Death-Ligand 1 (PD-L1) axis has demonstrated clinical activity in esophageal squamous cell carcinoma (ESCC). However, therapeutic responses remain limited in a substantial proportion of patients. Metabolic reprogramming and lactate accumulation are hallmarks of the tumor microenvironment and have been implicated in immune suppression, yet the epigenetic mechanisms linking lactate metabolism to immune checkpoint regulation in ESCC remain poorly understood. The aim of this study was to investigate whether lactate-induced histone lactylation regulates PD-L1 expression and contributes to tumor-intrinsic PD-L1 regulation in ESCC.

Methods: ESCC cell lines were treated with exogenous lactate and metabolic modulators to evaluate PD-L1 expression and histone H3 lysine 18 lactylation (H3K18la). The involvement of the lactyltransferase p300 was assessed using pharmacological inhibition. T cell function was examined using an *in vitro* co-culture system with activated Jurkat T cells. *In vivo* validation was performed using a nude mouse xenograft model, followed by immunohistochemical analysis.

Results: Lactate treatment significantly increased PD-L1 expression in ESCC cells ($p < 0.001$), accompanied by elevated global H3K18la ($p < 0.001$). Inhibition of glycolysis reduced basal H3K18la and PD-L1 expression ($p < 0.001$), which was restored by exogenous lactate ($p < 0.001$). Pharmacological inhibition of p300 abrogated lactate-induced H3K18la and PD-L1 upregulation ($p < 0.001$), indicating a p300-dependent mechanism. Functionally, lactate-treated ESCC cells reduced the expression of the activation-associated cytokines Interleukin-2 (IL-2) and Interferon-gamma (IFN-γ) in co-cultured Jurkat T cells ($p < 0.001$), indicative of attenuated T-cell activation, an effect partially reversed by PD-L1 knockdown. *In vivo*, lactate administration increased intratumoral H3K18la and PD-L1 expression in xenograft tumors ($p < 0.001$).

Conclusions: Our findings identify a lactate-p300-H3K18 lactylation axis associated with transcriptional upregulation of PD-L1 and attenuation of T-cell activation in a Jurkat-based co-culture system, providing a metabolic-epigenetic framework for improving immunotherapeutic strategies.

Keywords: esophageal squamous cell carcinoma; lactate; histone lactylation; H3K18 lactylation; PD-L1; immunosuppression

Introduction

Esophageal cancer is one of the most aggressive malignancies in the world, ranking sixth among causes of cancer-related mortality [1,2]. The predominant histological subtype is esophageal squamous cell carcinoma (ESCC), particularly in East Asia [3,4]. Despite advances in multimodal therapies, including surgery, chemoradiotherapy, and targeted therapy, the 5-year survival rate for patients with advanced ESCC remains dismal [5,6]. Recently, immune checkpoint blockade (ICB) therapy targeting the Programmed Death-1 (PD-1)/Programmed Death-Ligand 1 (PD-L1) axis has emerged as a promising treatment strategy [7,8,9]. However, only a fraction of ESCC patients derive durable clinical benefits from immunotherapy [10]. The variability in treatment response is largely attributed to the complex immunosuppressive tumor microenvironment (TME), which facilitates immune evasion [11]. Therefore, elucidating the molecular mechanisms regulating PD-L1 expression and identifying strategies to overcome the immunosuppressive TME are urgent unmet needs for improving treatment outcomes in ESCC.

Metabolic reprogramming is a hallmark of cancer [12,13,14]. Unlike normal cells, cancer cells preferentially produce energy through glycolysis even under oxygen-sufficient conditions, a phenomenon known as the "Warburg effect" [15,16]. This metabolic shift results in the massive accumulation of lactate within the TME [17,18]. Historically, lactate was viewed merely as a metabolic waste

product. However, accumulating evidence suggests that it functions as a potent signaling molecule modulating immune responses [19]. High concentrations of lactate in the TME have been reported to inhibit the function of natural killer (NK) cells and cytotoxic T lymphocytes (CTLs) while promoting the expansion of myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs) [20]. While acidification of the tumor microenvironment is a well-established mechanism, whether lactate exerts intrinsic regulatory effects on tumor cells to promote immune checkpoint expression remains poorly understood [21,22].

Recent breakthroughs in epigenetics have revealed a direct link between cellular metabolism and gene regulation. Zhang et al. [23] identified a novel histone modification termed "lactylation" in 2019, wherein lactate-derived lactyl groups are added to histone lysine residues, thereby stimulating gene transcription. This result demonstrated that lactate can serve as an epigenetic substrate to regulate chromatin structure and gene expression. Specifically, histone H3 lysine 18 lactylation (H3K18la) has been identified as a critical modification driven by the acetyltransferase p300, which acts as a lactyltransferase under high-lactate conditions [24]. Although histone lactylation has been implicated in macrophage polarization and tumorigenesis in other contexts, its specific role in regulating the immune checkpoint PD-L1 in ESCC has not been explored.

Despite accumulating evidence that tumor-derived lactate contributes to immune suppression within the tumor microenvironment, the epigenetic mechanisms linking metabolic reprogramming to the regulation of immune checkpoints in ESCC remain poorly understood [17,25]. In particular, whether histone lactylation directly participates in the transcriptional control of immune checkpoint molecules has not been elucidated. Moreover, the specific histone lactylation sites involved, the regulatory enzymes responsible for this modification, and its functional consequences on antitumor T cell responses in ESCC have yet to be well defined.

In this study, we systematically investigated the role of lactate-induced histone lactylation in regulating PD-L1 expression and immune evasion in ESCC. Specifically, we demonstrate that elevated lactate enhances PD-L1 transcription through H3K18 lactylation in a p300-dependent manner. Functionally, lactate-induced PD-L1 expression attenuates T-cell activation, as reflected by reduced Interleukin-2 (IL-2) and Interferon-gamma (IFN-γ) expression in co-cultured Jurkat T cells, while genetic or pharmacological disruption of this pathway partially restores these activation markers *in vitro*. Our findings reveal a mechanistic link between tumor metabolic reprogramming, epigenetic modification, and PD-L1-mediated immunosuppression in ESCC, providing a rationale for targeting lactate-driven histone lactylation to improve immunotherapeutic efficacy.

Materials and Methods

Cell Lines and Cell Culture

Human ESCC cell lines KYSE-30 (Cat. No. TCHu266) and TE-1 (Cat. No. TCHu89) were obtained from the Chinese Academy of Sciences Cell Bank (Shanghai, China). The Jurkat T-cell leukemia cell line was purchased from ATCC (Cat. TIB-152, Manassas, VA, USA). Cells were cultured in RPMI-1640 medium (Cat. 11875093, Gibco, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with fetal bovine serum (FBS, 10%; Cat. 10099141, Gibco) and penicillin-streptomycin (1%; Cat. 15140122, Gibco). Cells were maintained in a humidified incubator at 37 °C with 5% CO_2. The cells were authenticated using Short Tandem Repeat (STR) analysis and confirmed to be free of mycoplasma.

Lactate Treatment and Metabolic Modulation

Sodium L-lactate (L-Lactate, Cat. L7022), 2-Deoxy-D-glucose (2-DG, Cat. D8375), and the p300 inhibitor C646 (Cat. SML0002) were purchased from Sigma-Aldrich (St. Louis, MO, USA). ESCC cells were seeded in 6-well plates. Upon reaching 60–70% confluence, the medium was replaced with fresh medium containing L-lactate (0, 5, 10, and 20 mM) for 24 hours. Lactate was dissolved in the culture medium, and the pH was adjusted to 7.4 with NaOH to exclude the influence of acidic pH. For mechanistic validation, cells were treated with the glycolysis inhibitor 2-DG (5 mM) or the p300 inhibitor C646 (10 μM) either alone or in combination with lactate (20 mM) for 24 hours.

Transfection of Small Interfering RNA (siRNA)

siRNAs targeting human *CD274* (PD-L1) and the negative control siRNA (si-NC) were synthesized by GenePharma (Shanghai, China). Cells were transfected with siRNAs using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) at a final siRNA concentration of 50 nM. The transfection efficiency was verified by qPCR 48 hours post-transfection. Sequences of siRNAs used are listed in Table 1. The knockdown efficiency of si-*PD-L1* was confirmed by qPCR analysis of *CD274* mRNA in transfected ESCC cells prior to co-culture.

RNA Extraction and Quantitative Real-Time PCR (qPCR)

Total RNA was extracted from ESCC cells or Jurkat T cells using the TRIzol reagent (Cat. 15596026, Invitrogen, Waltham, MA, USA). Reverse transcription was performed using the PrimeScript™ RT Reagent Kit (Cat. RR037A, Takara, Shiga, Japan) to synthesize cDNA. qPCR was conducted using SYBR Green Premix Ex Taq (Cat. RR420A, Takara) on a Real-Time PCR System (ABI 7500, Applied Biosystems, Foster City, CA, USA). Relative levels of mRNA were calculated using the $2^{-\Delta\Delta Ct}$ method, with

Table 1. Sequences of siRNAs used in this study.

Name	Direction	Sequence (5′ to 3′)
si-*PD-L1* #1	Sense	UCU AAU UGU UUU UCU ACU GGG dTdT
	Antisense	CAG UAG AAA AAC AAU UAG ACC dTdT
si-*PD-L1* #2	Sense	UAU UGC UAC CAU ACU CUA CCA dTdT
	Antisense	GUA GAG UAU GGU AGC AAU AUG dTdT
si-NC	Sense	UUC UCC GAA CGU GUC ACG U dTdT
	Antisense	ACG UGA CAC GUU CGG AGA A dTdT

PD-L1, Programmed Death-Ligand 1; si-NC, negative control siRNA.

Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) as the internal control. The primer sequences are listed in Table 2.

Table 2. Primer sequences.

Primer	Sequence (5′-3′)
hum-*PD-L1*-F	AGGGCATTCCAGAAAGATGAGG
hum-*PD-L1*-R	TGTATGGGGCGTTCAGCAAA
hum-*GAPDH*-F	GTGGATATTGTTGCCATCAATGACC
hum-*GAPDH*-R	GCCCCAGCCTTCTTCATGGTGGT
hum-*IFN-γ*-F	TGTCGCCAGCAGCTAAAACA
hum-*IFN-γ*-R	TGCAGGCAGGACAACCATTA
hum-*IL-2*-F	AACCTCAACTCCTGCCACAA
hum-*IL-2*-R	GCATCCTGGTGAGTTTGGGA

GAPDH, Glyceraldehyde-3-phosphate dehydrogenas; F, forward; R, reverse; *IFN-γ*, Interferon-gamma; *IL-2*, Interleukin-2.

Western Blot Analysis

Cells were lysed in RIPA lysis buffer (Cat. P0013B, Beyotime, Shanghai, China) containing protease and phosphatase inhibitors (PMSF, Cat. ST506, Beyotime). A nuclear protein extraction kit (Cat. P0027, Beyotime, Shanghai, China) was used for high-yield histone extraction. Protein concentration was determined using a BCA Protein Assay Kit (Cat. 23225, Thermo Fisher Scientific, Rockford, IL, USA). Proteins (20–30 µg) were separated by 10–12% SDS-PAGE and transferred onto PVDF membranes (Cat. IPVH00010, Merck Millipore, Burlington, MA, USA). After blocking with 5% skim milk for 1 hour at room temperature, membranes were incubated (1:1000) with primary antibodies of GAPDH (TA-08, ZS Golden Bridge, Beijing, China), PD-L1 (ET1701-41, HUABIO, Hangzhou, China), H3 (ET1701-64, HUABIO), and H3k18la (Abs174920, Absin, Shanghai, China) overnight at 4 °C, followed by incubation with HRP-conjugated secondary antibodies (1:2000; ZB-2301/ZB-2305, ZS Golden Bridge) for 1 hour. Protein bands were visualized using the ECL detection system (Cat. 34577, Thermo Fisher Scientific) and quantified using ImageJ software (version 1.53, NIH, Bethesda, MD, USA).

In Vitro Co-Culture Assay

ESCC cells were seeded in 24-well plates and pretreated with lactate (20 mM) or transfected with si-PD-L1 as described above. Before co-culture, Jurkat T cells were activated with Phorbol 12-myristate 13-acetate (PMA, 50 ng/mL, Cat. P8139, Sigma-Aldrich, St. Louis, MO, USA) and Ionomycin (1 µg/mL, Cat. I0634, Sigma-Aldrich) for 6 hours. The culture medium was removed, and the ESCC cells were washed with PBS to eliminate residual lactate. Activated Jurkat cells were then added to the ESCC monolayer at a ratio of 5:1 (Effector:Target) and co-cultured for 24 hours. After co-culture, Jurkat T cells were collected by centrifugation and subjected to qPCR analysis of *IL-2* and *IFN-γ* expression. Tumor cell morphology and T-cell aggregation were observed and photographed under an inverted microscope (CKX53, Olympus, Tokyo, Japan).

Tumor Xenograft Model

Female BALB/c nude mice (6 weeks old) were obtained from Vital River (Beijing, China) and housed under specific pathogen-free (SPF) conditions. The animal study was approved by the Animal Ethics Committee of Guangdong Medical University (Approval No. 2026030003). All animal experiments were performed in accordance with the ARRIVE 2.0 guidelines and the institutional guidelines for the care and use of laboratory animals. KYSE-30 ESCC cells (5×10^6) suspended in 100 µL PBS were injected subcutaneously into the right flank of the mice. Mice were randomly divided into two groups (n = 4 per group): Control (PBS) and Lactate (2 g/kg, intraperitoneal injection, every other day). Tumor volume was measured weekly and calculated as Volume = $0.5 \times \text{Length} \times \text{Width}^2$. After 4 weeks (28 days) of treatment, mice were deeply anesthetized with pentobarbital sodium (50 mg/kg, i.p., P3761, Sigma-Aldrich, St. Louis, MO, USA) and sacrificed by decapitation. Tumors were then excised, measured, weighed, and processed for histological analysis.

Immunohistochemistry (IHC)

After fixation in 4% paraformaldehyde and paraffin embedding, tumor tissues were sectioned at a thickness of 4 µm. Sections were deparaffinized, rehydrated, and subjected to antigen retrieval in citrate buffer (pH

7.4). After blocking endogenous peroxidase and non-specific binding, sections were incubated with anti-PD-L1 (1:1000; GB115736-100, Servicebio, Wuhan, China) and anti-H3K18la (1:1000; abs174920, Absin) antibodies overnight at 4 °C. Sections were then incubated with a secondary antibody (1:1000; GB23301/GB23303, Servicebio) and stained with DAB (PA140212, TIANGEN, Beijing, China) substrate. Hematoxylin was used for counterstaining. Images were captured using a microscope (Nikon Eclipse Ci, Nikon, Tokyo, Japan) under identical exposure settings. H3K18la and PD-L1 staining were quantified using ImageJ (NIH, Bethesda, MD, USA) and expressed as a percentage of the DAB-positive area relative to the total tissue area. Quantification was performed independently by two observers blinded to group allocation, each analyzing at least five randomly selected, non-overlapping fields per section, and the mean value was used for analysis.

Statistical Analysis

All *in vitro* experiments were repeated five times independently. Data are presented as mean ± standard deviation (SD). Statistical analysis was performed using GraphPad Prism 10.1.2 software (GraphPad Software, San Diego, CA, USA). Differences between two groups were analyzed using the Student's *t*-test. Comparisons among multiple groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. A p-value < 0.05 was considered statistically significant.

Results

Lactate Promotes PD-L1 Expression and H3K18 Lactylation in ESCC Cells

To investigate the potential link between metabolic reprogramming and immune checkpoint expression in ESCC, we first examined the effect of exogenous lactate on PD-L1 levels. Two ESCC cell lines, KYSE-30 and TE-1, were treated with increasing concentrations of L-lactate (0, 5, 10, and 20 mM) for 24 hours. Western blot analysis revealed a significant, dose-dependent upregulation of PD-L1 protein expression in both cell lines (Fig. 1A,B).

Concurrently, given that lactate can serve as a substrate for histone lactylation, we assessed the levels of histone H3K18la. As expected, lactate treatment markedly increased global H3K18la levels, which positively correlated with the upregulation of PD-L1 (Fig. 1A,B). Quantitative analysis confirmed that the induction of both PD-L1 ($p < 0.001$) and H3K18la ($p < 0.001$) was most prominent at the concentration of 20 mM (Fig. 1C,D). Based on these findings, 20 mM lactate was selected for subsequent experiments.

Lactate Regulates PD-L1 Expression at the Transcriptional Level via Glycolysis-Dependent Metabolism

Since both KYSE-30 and TE-1 cell lines exhibited comparable, dose-dependent upregulation of PD-L1 and H3K18la in response to lactate (Fig. 1), KYSE-30 was selected as a representative ESCC cell line for the subsequent mechanistic and functional experiments. We next sought to determine whether lactate regulates PD-L1 expression at the transcriptional level and whether this process depends on endogenous lactate production through aerobic glycolysis. To this end, we treated cells with 2-Deoxy-D-glucose (2-DG), a glycolysis inhibitor, to deplete intracellular lactate, followed by supplementation with exogenous lactate (20 mM) as a rescue. 2-DG treatment significantly reduced *CD274* mRNA levels compared to the control ($p < 0.001$), whereas supplementation with exogenous lactate restored and elevated *CD274* expression (2-DG vs 2-DG+Lactate, $p < 0.001$; Control vs 2-DG+Lactate, $p < 0.001$), suggesting that lactate modulates PD-L1 at the transcriptional level (Fig. 2A). Consistently, Western blot analysis revealed that 2-DG led to a substantial reduction of both H3K18la and PD-L1, which were not only restored but significantly exceeded basal levels upon exogenous lactate supplementation (Fig. 2B,C, all $p < 0.001$). These results suggest that lactate is sufficient to restore H3K18 lactylation and PD-L1 expression under glycolytic inhibition, supporting its key role in driving H3K18la and subsequent PD-L1 upregulation. Whether lactate directly serves as the lactyl-group donor at this site, and whether other glycolytic intermediates also contribute, remains to be established.

p300 Mediates Lactate-Induced H3K18 Lactylation and PD-L1 Upregulation

Previous studies have identified p300 as a key "writer" enzyme responsible for histone lactylation [23]. To explore the mechanistic role of p300 in our model, we utilized C646, a specific p300 inhibitor. ESCC cells were treated with lactate (20 mM) with or without C646.

C646 treatment significantly abrogated the lactate-induced elevation of H3K18la (Fig. 3C, $p < 0.001$). Consistently, the upregulation of PD-L1 protein induced by lactate was also blocked by C646 (Fig. 3B, $p < 0.001$). Furthermore, qPCR results confirmed that p300 inhibition prevented the lactate-induced increase in *CD274* mRNA levels (Fig. 3A, $p < 0.001$). Collectively, these data suggest that lactate promotes PD-L1 transcription in a p300-dependent manner, in association with increased H3K18 lactylation. As C646 inhibits p300 activity broadly rather than H3K18la specifically, and direct enrichment of H3K18la at the *CD274* locus was not assessed, the extent to which H3K18 lactylation directly mediates *CD274* transcription remains to be established.

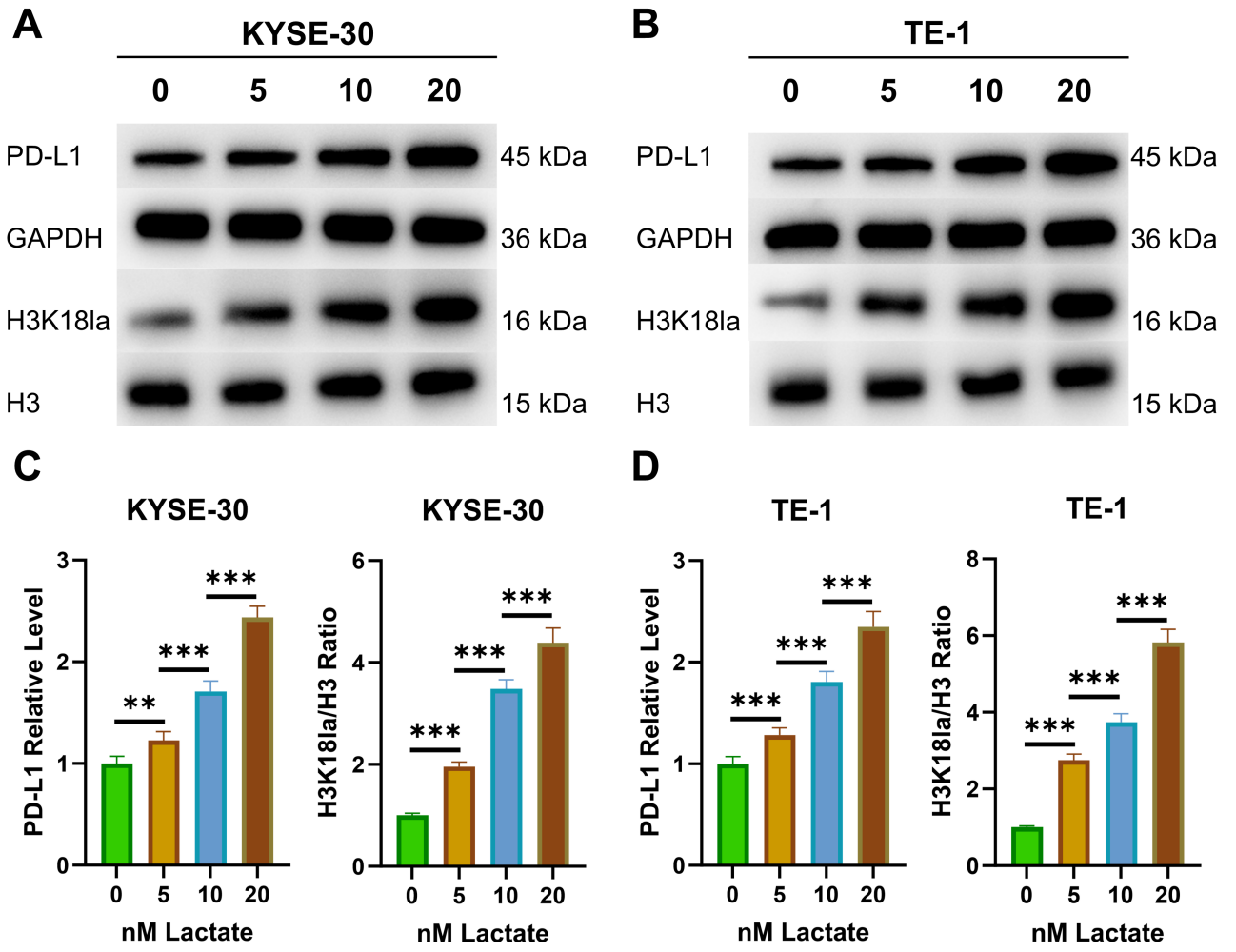

Fig. 1. Lactate upregulates PD-L1 expression and histone lactylation in ESCC cells. (A,B) Western blot analysis of PD-L1 and H3K18la expression in KYSE-30 (A) and TE-1 (B) cells treated with lactate. Histone H3 and GAPDH were used as loading controls for nuclear and cytoplasmic proteins, respectively. (C,D) Quantitative analysis of PD-L1 and H3K18la protein levels relative to controls. The band intensities were normalized to GAPDH (for PD-L1) or Histone H3 (for H3K18la). Data are presented as mean ± SD (n = 5). $^{**}p < 0.01$, $^{***}p < 0.001$. ESCC, esophageal squamous cell carcinoma; H3K18la, H3 lysine 18 lactylation; SD, standard deviation.

Lactate-Induced PD-L1 Attenuates T-Cell Activation In Vitro

To evaluate the functional consequence of lactate-induced PD-L1 upregulation on T-cell activation, we established a co-culture system using ESCC cells and activated Jurkat T cells. ESCC cells were pre-treated with lactate and/or transfected with specific siRNA targeting *PD-L1* (si-*PD-L1*) prior to co-culture. Two independent siRNAs targeting *CD274* (si-*PD-L1* #1 and si-*PD-L1* #2) were evaluated by qPCR. Both siRNAs reduced *PD-L1* mRNA (all $p < 0.001$), and si-*PD-L1* #1 showed the higher knockdown efficiency (Fig. 4A). Therefore, si-*PD-L1* #1 was selected for all subsequent co-culture experiments.

We observed that Jurkat T cells co-cultured with lactate-pretreated ESCC cells exhibited significantly reduced mRNA expression of the activation-associated cytokines *IFN-γ* and *IL-2* compared to the control (si-NC) group, indicative of attenuated T-cell activation (Fig. 4B,C, $p < 0.001$). However, this effect was partially reversed when PD-L1 was knocked down in the lactate-treated ESCC cells (Fig. 4B,C, $p < 0.001$).

Morphological analysis further supported these findings (Fig. 4D). In the control group, T cells formed clusters and adhered to tumor cells. In contrast, in the lactate-treated group, T cells remained dispersed and suspended, while tumor cells maintained a healthy, adherent morphology, consistent with reduced T cell clustering and adhesion in this co-culture system. Notably, silencing PD-L1 restored T cell clustering and adhesion, accompanied by morphological changes in the tumor cell monolayer even in the presence of lactate. As these observations are based on light-microscopic morphology and were not accompanied by antigen-specific recognition, cytotoxicity, or cell-death assays, they should be interpreted as descriptive differences

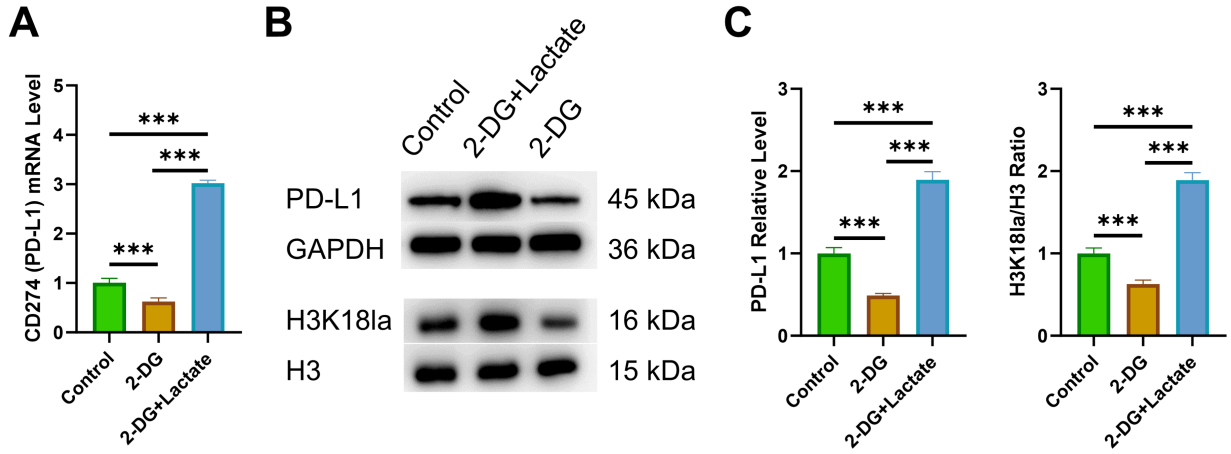

Fig. 2. Lactate regulates PD-L1 expression at the transcriptional level via glycolysis-dependent metabolism. (A) The mRNA levels of *CD274* (PD-L1) in KYSE-30 cells treated with control medium, the glycolysis inhibitor 2-DG (5 mM), or 2-DG (5 mM) combined with lactate (20 mM), measured by qPCR. (B) Representative Western blot analyses of PD-L1 and H3K18la in KYSE-30 cells under the same treatments. Note that the lanes were loaded in the order Control, 2-DG+Lactate, and 2-DG, as indicated above each lane. This order differs from that of the bar graph in panels (A) and (C), so please refer to the lane labels rather than assuming a left-to-right correspondence. (C) Quantification of the band intensities in (B), presented in the order Control, 2-DG, and 2-DG+Lactate. Data are presented as mean ± SD (n = 5). ***$p < 0.001$. 2-DG, 2-Deoxy-D-glucose; qPCR, Quantitative Real-Time PCR.

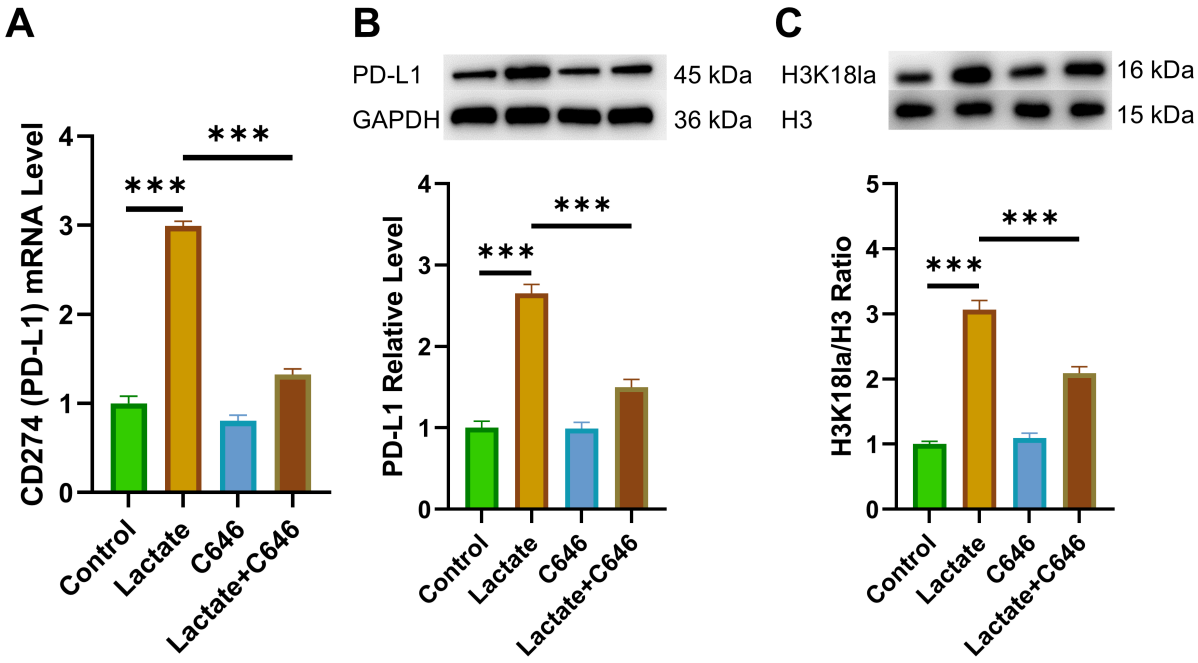

Fig. 3. p300 mediates lactate-induced H3K18 lactylation and PD-L1 upregulation. (A) qPCR analysis of *CD274* mRNA expression levels in KYSE-30 cells treated with lactate (20 mM) in the presence or absence of the p300 inhibitor C646 (10 μM). (B,C) Quantitative analysis of PD-L1 (B) and H3K18la (C) protein levels. Data are presented as mean ± SD (n = 5). ***$p < 0.001$.

in T cell-tumor cell interaction rather than direct evidence of immune-mediated tumor cell killing. These results indicate that lactate attenuates Jurkat T-cell activation at least in part through the upregulation of the PD-L1 immune checkpoint.

Lactate Promotes H3K18la and PD-L1 Expression In Vivo

Finally, to validate *in vitro* findings in a physiological context, we established a xenograft tumor model in nude mice. Mice bearing ESCC tumors were treated with intraperitoneal injections of lactate or PBS (Ctrl).

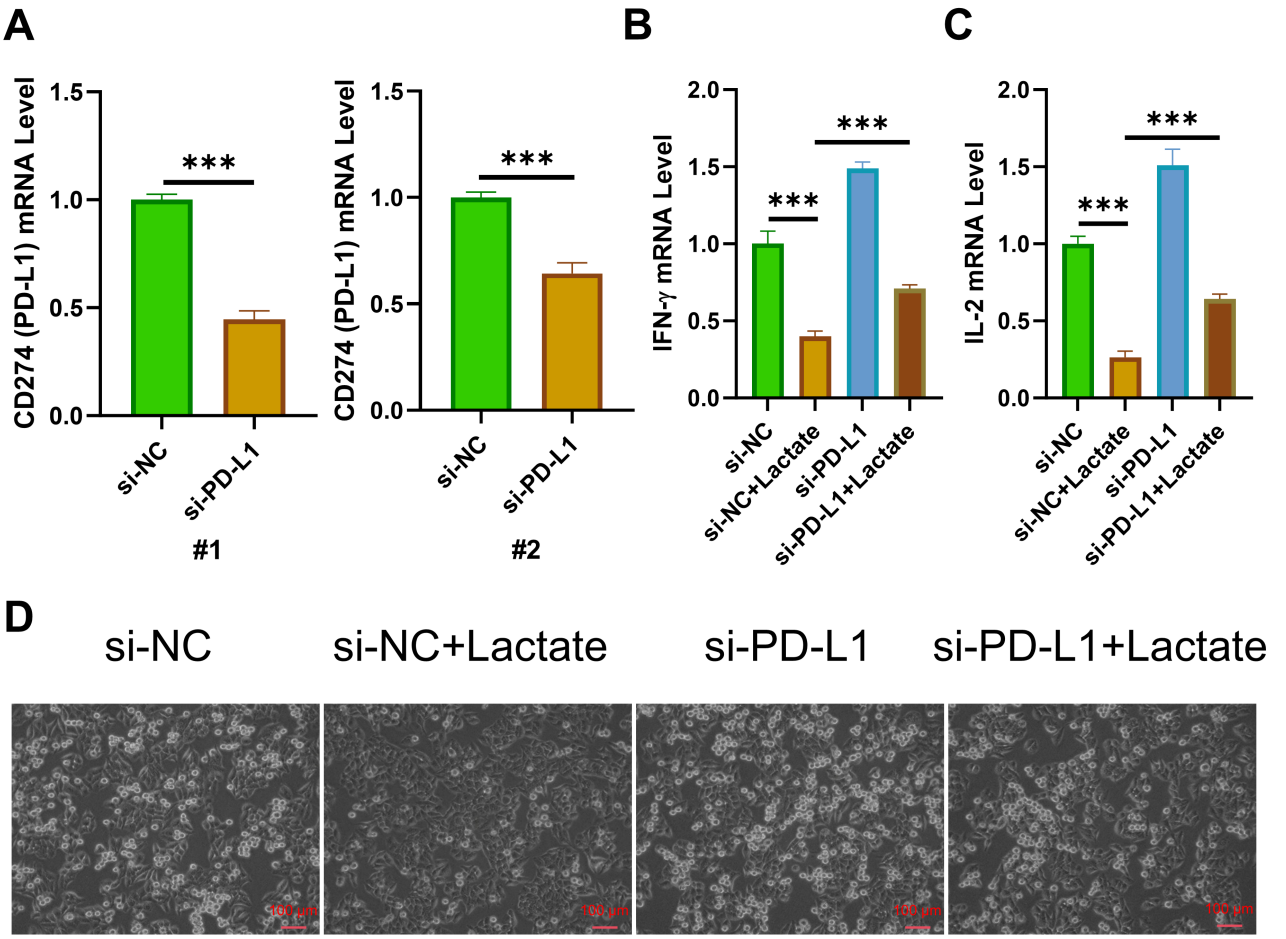

Fig. 4. Lactate-induced PD-L1 attenuates T-cell activation *in vitro*. (A) The knockdown efficiency of si-*PD-L1* was verified by qPCR analysis of *CD274* (PD-L1) mRNA levels in KYSE-30 cells. si-*PD-L1* #1, which showed higher knockdown efficiency, was used for the subsequent functional co-culture experiments. (B,C) The mRNA levels of *IFNG* (IFN-γ) (B) and *IL2* (IL-2) (C) in Jurkat T cells were detected by qPCR. (D) Representative microscopic images of the co-culture system. The control group showed T cell clustering and adhesion to tumor cells. The Lactate group exhibited dispersed T cells and confluent, adherent tumor cells, while PD-L1 knockdown restored T cell aggregation, accompanied by morphological changes in the tumor cell monolayer. Scale bar: 100 μm. Data are presented as mean ± SD (n = 5). ***$p < 0.001$.

As shown in Fig. 5A, the lactate-treated group exhibited accelerated tumor growth compared to the control group across all animals (n = 4 per group), with larger tumor volumes and weights presented for each mouse (Fig. 5A, all $p < 0.001$). Immunohistochemical (IHC) staining revealed significantly stronger staining intensity for both H3K18la and PD-L1 in tumors from lactate-treated mice (Fig. 5B, all $p < 0.001$). Quantitative analysis of the positive-staining area confirmed a positive correlation between lactate treatment and upregulation of these markers *in vivo*. These data corroborate the "Lactate-H3K18la-PD-L1" axis in tumor tissues *in vivo*.

Discussion

In the present study, we elucidated a novel mechanism linking metabolic reprogramming to tumor-intrinsic PD-L1 regulation in ESCC. We demonstrated that elevated exogenous lactate acts not merely as a metabolic waste product but as a pivotal signaling molecule that upregulates PD-L1 expression. Mechanistically, we identified that lactate promotes histone H3K18la in a p300-dependent manner, which in turn activates *CD274* (PD-L1) transcription. Furthermore, our *in vitro* co-culture assays suggested that lactate-induced PD-L1 attenuates the activation of co-cultured Jurkat T cells *in vitro*. Importantly, our *in vivo* findings supported the activity of the "Lactate-H3K18la-PD-L1" axis in an immunodeficient xenograft model.

The Warburg effect is characterized by enhanced aerobic glycolysis and lactate production, and is a hallmark of cancer [26,27,28]. Historically, lactate was viewed as a metabolic byproduct. However, the discovery of histone lactylation has redefined it as an epigenetic modifier

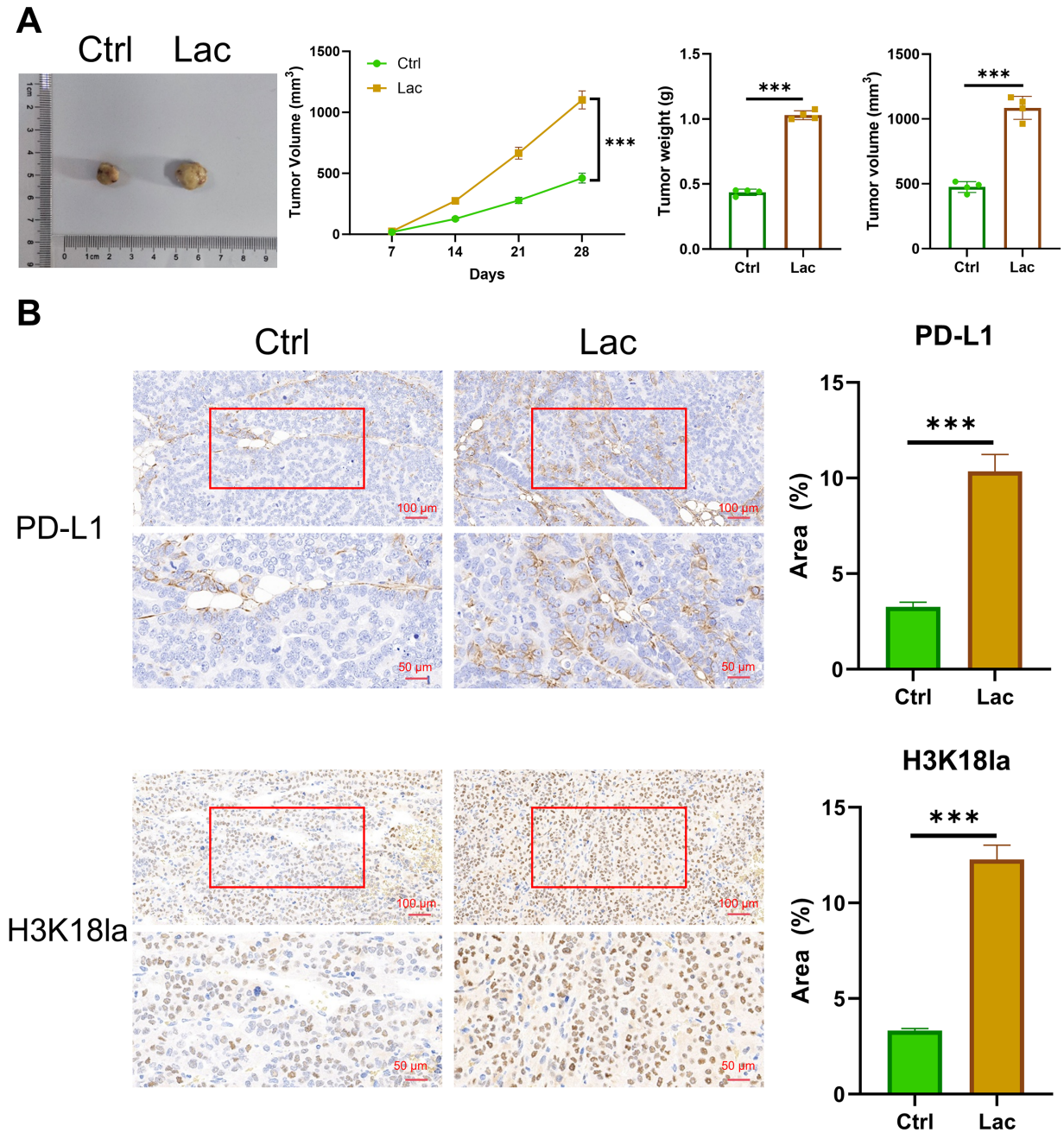

Fig. 5. Lactate promotes tumor growth and upregulates H3K18la and PD-L1 *in vivo*. (A) Representative images of subcutaneous tumors excised from nude mice treated with PBS (Ctrl) or Lactate (Lac), n = 4 per group. Tumor growth curves are presented as mean ± SD at the indicated time points (days 7, 14, 21, and 28). Endpoint tumor volume and tumor weight are shown for all individual animals, with each dot representing one mouse and bars indicating mean ± SD. Error bars represent SD; where not visible, the SD is smaller than the size of the symbol. (B) Representative immunohistochemistry (IHC) images of H3K18la and PD-L1 staining in tumor tissues from the indicated groups, and quantitative analysis of H3K18la and PD-L1 staining area. Lactate treatment significantly increased the levels of H3K18la and PD-L1 in tumor tissues. Boxed areas indicate magnified regions. Data are presented as mean ± SD (n = 4). ***$p <$ 0.001. PBS, Phosphate-buffered saline.

[23]. Our findings extend this concept to ESCC, showing that high lactate concentrations drive H3K18 lactylation. We observed that pharmacological inhibition of glycolysis using 2-DG reduced basal H3K18la and PD-L1 levels, which could be rescued by exogenous lactate. This suggests a direct coupling between the metabolic state of the tumor and its epigenetic landscape. Consistent with previous reports identifying p300 as a lactyltransferase

[29], we confirmed that p300 inhibition (C646) abrogated lactate-induced H3K18la and PD-L1 expression. These data highlight a therapeutic vulnerability where targeting the metabolic-epigenetic axis, specifically p300 or lactate transport, might sensitize ESCC to immunotherapy.

PD-L1 expression in cancer cells is known to be regulated by multiple oncogenic and inflammatory signaling pathways, including IFN-γ-Janus kinase (JAK)/Signal transducer and activator of transcription (STAT) signaling, Nuclear factor kappa B (NF-κB) activation, and hypoxia-driven Hypoxia-inducible factor 1 alpha (HIF-1α) signaling [30,31]. These pathways predominantly respond to extrinsic immune cues or microenvironmental stress and converge on transcriptional activation of the *CD274* gene [30,32]. However, how intrinsic metabolic alterations within tumor cells interface with these established regulatory networks remains incompletely understood.

Our findings suggest that lactate-driven histone lactylation represents a metabolically induced epigenetic mechanism that may operate in parallel with, or upstream of, classical PD-L1 regulatory pathways. Unlike cytokine-dependent signaling, lactate accumulation reflects a cell-intrinsic consequence of aerobic glycolysis and directly modifies chromatin structure via p300-mediated H3K18 lactylation [29,33]. This modification may increase chromatin accessibility at immune checkpoint-related loci, thereby lowering the threshold for PD-L1 transcriptional activation. Notably, such a mechanism could help explain the elevated basal PD-L1 expression observed in highly glycolytic tumors even in the absence of strong inflammatory stimulation. These observations together position histone lactylation as an additional regulatory layer that integrates metabolic state with immune checkpoint control.

The upregulation of PD-L1 is a well-established mechanism for tumor immune evasion [34]. Our study provides evidence that the metabolic microenvironment directly regulates tumor-intrinsic PD-L1 expression. In our co-culture system, lactate-pretreated ESCC cells exhibited an inhibitory effect on Jurkat T cells, characterized by reduced *IL2* and *IFNG* expression and impaired T-cell clustering. Notably, silencing PD-L1 partially reversed these immunosuppressive effects even in the presence of lactate. This result is crucial as it indicates that, although lactate may exert broad immunosuppressive effects (e.g., through acidification), H3K18la-mediated PD-L1 upregulation contributes to reduced T-cell activation in this *in vitro* context.

We employed a xenograft model in nude mice to physiologically validate our findings. We observed that lactate administration significantly promoted tumor growth and increased intratumoral levels of H3K18la and PD-L1. It is important to note that BALB/c nude mice are T-cell deficient [35] and therefore, the accelerated tumor growth observed in this model is likely attributable to the intrinsic metabolic advantages provided by lactate or other oncogenic signaling pathways, rather than T-cell-mediated immune escape. However, the pivotal contribution of our *in vivo* data lies in the confirmation of the molecular mechanism by which lactate treatment successfully created a hyper-lactylated chromatin environment and sustained high PD-L1 protein levels in the tumor tissues. These findings serve as a proof of concept that the "Lactate-H3K18la-PD-L1" signaling axis is operative *in vivo*. Whether this PD-L1 upregulation contributes to T-cell suppression *in vivo* cannot be determined in this immunodeficient model and requires further validation using immunocompetent animal models.

From a translational perspective, our study highlights multiple potential therapeutic strategies to target the lactate-driven, tumor-intrinsic PD-L1 regulation in ESCC. Given that lactate accumulation is a hallmark of tumor glycolysis [36], targeting lactate production through glycolytic inhibition (e.g., lactate dehydrogenase A [LDHA] inhibition) or limiting lactate export via monocarboxylate transporter (MCT) blockade may reduce histone lactylation and subsequent PD-L1 upregulation. In addition, pharmacological inhibition of p300 provides a more direct approach to suppress lactylation-dependent transcriptional activation of immune checkpoint genes.

Importantly, these metabolic or epigenetic interventions may be particularly effective when combined with immune checkpoint blockade. By lowering tumor-intrinsic PD-L1 expression and alleviating metabolic immunosuppression, targeting the lactate-p300-histone lactylation axis could potentially sensitize ESCC tumors to PD-1/PD-L1-based immunotherapy. This combinatorial strategy may help overcome primary resistance associated with high glycolytic activity and immune-excluded tumor phenotypes, offering a rationale for future preclinical and clinical evaluation.

There are still several limitations in this study. Our data support a critical role for H3K18 lactylation in regulating PD-L1 expression; however, direct evidence of H3K18 lactylation enrichment at the *CD274* promoter or enhancer regions requires further validation using chromatin immunoprecipitation-based approaches. In addition, 2-DG and C646 are pharmacological tools whose effects may not be entirely specific to glycolytic inhibition and p300 inhibition, respectively, and complementary genetic approaches will be needed to corroborate the specificity of these findings. The mechanistic experiments were also performed predominantly in KYSE-30 cells, and this limited number of cell lines may not fully capture the molecular heterogeneity of ESCC, so validation across a broader panel of ESCC cell lines is warranted. Furthermore, PD-L1 knockdown was confirmed primarily at the mRNA level, whereas validation at the membrane protein level (e.g., by flow cytometry of cell-surface PD-L1) was lacking. Such analyses would provide a more direct link between the observed functional effects and surface PD-L1 abundance. It should also be noted that the Jurkat cell line is an immortalized leukemic T-cell line, and the IL-2 and IFN-γ mRNA lev-

els measured in this study reflect T-cell activation as a surrogate readout rather than direct cytotoxic killing. Therefore, our co-culture data should be interpreted as *in vitro* evidence of attenuated T-cell activation rather than definitive proof of anti-tumor immune evasion. Validation using primary human $CD8^+$ T cells, antigen-specific cytotoxicity assays, and immunocompetent or humanized models will be required to establish the immunological relevance of this pathway. Due to the use of immunodeficient mouse models, the *in vivo* contribution of lactate-driven histone lactylation to antitumor T cell immunity could not be fully assessed. In addition, the fixed concentrations of exogenously administered lactate and the intraperitoneal route of delivery may not fully recapitulate the dynamic, spatially heterogeneous lactate gradients within the native tumor microenvironment, where lactate is continuously generated and locally enriched by glycolytic tumor cells. Moreover, the animal experiments were conducted with a relatively small sample size, and the semi-quantitative IHC scoring used in this study differs from the standardized PD-L1 scoring systems used in clinical practice, such as the combined positive score, thereby limiting direct translational comparison. Future studies employing immunocompetent or humanized mouse models will be essential to evaluate the immunological relevance of this pathway *in vivo*.

In addition, extending these findings to clinical ESCC specimens by correlating intratumoral lactate levels, histone lactylation status, and PD-L1 expression with patient outcomes may further strengthen the translational significance of this work. Together, these efforts will help clarify the broader impact of metabolic-epigenetic crosstalk on tumor immunity and guide the development of rational combination strategies integrating metabolic modulation with immunotherapy.

Conclusions

In conclusion, our study suggests a potential regulatory axis wherein lactate acts via p300 to induce H3K18 lactylation, thereby transcriptionally upregulating tumor-intrinsic PD-L1 in ESCC. These metabolic-epigenetic changes may contribute to alterations in immune-related signaling, providing a theoretical rationale for targeting lactate metabolism or histone lactylation modifiers as a strategy to enhance immunotherapy efficacy in esophageal cancer. As the immune-related effects were largely derived from *in vitro* assays and an immunodeficient *in vivo* model, the immunological relevance of this axis requires further validation using immunocompetent animal models.

Availability of Data and Materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions

TL and XJH designed the research study. TL, GHH and WSZ performed the research. XML, RL and XJH analyzed the data. TL drafted the article. All authors contributed to important editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The animal study was approved by the Animal Ethics Committee of Guangdong Medical University (approval number: 2026030003). All animal experiments were performed in accordance with the ARRIVE 2.0 guidelines.

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Conflict of Interest

The authors declare no conflict of interest.

References

[1] Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: A Cancer Journal for Clinicians. 2021; 71: 209–249. https://doi.org/10.3322/caac.21660

[2] Morgan E, Soerjomataram I, Rumgay H, Coleman HG, Thrift AP, Vignat J, et al. The Global Landscape of Esophageal Squamous Cell Carcinoma and Esophageal Adenocarcinoma Incidence and Mortality in 2020 and Projections to 2040: New Estimates From GLOBOCAN 2020. Gastroenterology. 2022; 163: 649–658.e2. https://doi.org/10.1053/j.gastro.2022.05.054

[3] Codipilly DC, Wang KK. Squamous Cell Carcinoma of the Esophagus. Gastroenterology Clinics of North America. 2022; 51: 457–484. https://doi.org/10.1016/j.gtc.2022.06.005

[4] Uhlenhopp DJ, Then EO, Sunkara T, Gaduputi V. Epidemiology of esophageal cancer: update in global trends, etiology and risk factors. Clinical Journal of Gastroenterology. 2020; 13: 1010–1021. https://doi.org/10.1007/s12328-020-01237-x

[5] Doki Y, Ajani JA, Kato K, Xu J, Wyrwicz L, Motoyama S, et al. Nivolumab Combination Therapy in Advanced Esophageal Squamous-Cell Carcinoma. The New England Journal of Medicine. 2022; 386: 449–462. https://doi.org/10.1056/NEJMoa2111380

[6] Yang H, Wang F, Hallemeier CL, Lerut T, Fu J. Oesophageal cancer. Lancet (London, England). 2024; 404: 1991–2005. https://doi.org/10.1016/S0140-6736(24)02226-8

[7] Sun JM, Shen L, Shah MA, Enzinger P, Adenis A, Doi T, et al. Pembrolizumab plus chemotherapy versus chemotherapy alone for first-line treatment of advanced oesophageal cancer (KEYNOTE-590): a randomised, placebo-controlled, phase 3

study. Lancet (London, England). 2021; 398: 759–771. https://doi.org/10.1016/S0140-6736(21)01234-4

[8] Shen L, Kato K, Kim SB, Ajani JA, Zhao K, He Z, et al. Tislelizumab Versus Chemotherapy as Second-Line Treatment for Advanced or Metastatic Esophageal Squamous Cell Carcinoma (RATIONALE-302): A Randomized Phase III Study. Journal of Clinical Oncology : Official Journal of the American Society of Clinical Oncology. 2022; 40: 3065–3076. https://doi.org/10.1200/JCO.21.01926

[9] Liu Q, Zhou P, Tian D. Efficacy and Safety of Neoadjuvant Chemoimmunotherapy in Patients With Muscle-Invasive Bladder Cancer. Discovery Medicine. 2025; 37: 1586–1594. https://doi.org/10.24976/Discov.Med.202537199.137

[10] Mimura K, Yamada L, Ujiie D, Hayase S, Tada T, Hanayama H, et al. Immunotherapy for esophageal squamous cell carcinoma: a review. Fukushima Journal of Medical Science. 2018; 64: 46–53. https://doi.org/10.5387/fms.2018-09

[11] Zheng Y, Chen Z, Han Y, Han L, Zou X, Zhou B, et al. Immune suppressive landscape in the human esophageal squamous cell carcinoma microenvironment. Nature Communications. 2020; 11: 6268. https://doi.org/10.1038/s41467-020-20019-0

[12] Pavlova NN, Zhu J, Thompson CB. The hallmarks of cancer metabolism: Still emerging. Cell Metabolism. 2022; 34: 355–377. https://doi.org/10.1016/j.cmet.2022.01.007

[13] Hanahan D. Hallmarks of Cancer: New Dimensions. Cancer Discovery. 2022; 12: 31–46. https://doi.org/10.1158/2159-8290.CD-21-1059

[14] Mitaishvili E, Feinsod H, David Z, Shpigel J, Fernandez C, Sauane M, et al. The Molecular Mechanisms behind Advanced Breast Cancer Metabolism: Warburg Effect, OXPHOS, and Calcium. Frontiers in Bioscience (Landmark Edition). 2024; 29: 99.https://doi.org/10.31083/j.fbl2903099

[15] DeBerardinis RJ, Chandel NS. We need to talk about the Warburg effect. Nature Metabolism. 2020; 2: 127–129. https://doi.org/10.1038/s42255-020-0172-2

[16] Wang Y, Patti GJ. The Warburg effect: a signature of mitochondrial overload. Trends in Cell Biology. 2023; 33: 1014–1020. https://doi.org/10.1016/j.tcb.2023.03.013

[17] Wang ZH, Peng WB, Zhang P, Yang XP, Zhou Q. Lactate in the tumour microenvironment: From immune modulation to therapy. EBioMedicine. 2021; 73: 103627. https://doi.org/10.1016/j.ebiom.2021.103627

[18] Apostolova P, Pearce EL. Lactic acid and lactate: revisiting the physiological roles in the tumor microenvironment. Trends in Immunology. 2022; 43: 969–977. https://doi.org/10.1016/j.it.2022.10.005

[19] Manoharan I, Prasad PD, Thangaraju M, Manicassamy S. Lactate-Dependent Regulation of Immune Responses by Dendritic Cells and Macrophages. Frontiers in Immunology. 2021; 12: 691134. https://doi.org/10.3389/fimmu.2021.691134

[20] Gu XY, Yang JL, Lai R, Zhou ZJ, Tang D, Hu L, et al. Impact of lactate on immune cell function in the tumor microenvironment: mechanisms and therapeutic perspectives. Frontiers in Immunology. 2025; 16: 1563303. https://doi.org/10.3389/fimmu.2025.1563303

[21] Huang ZW, Zhang XN, Zhang L, Liu LL, Zhang JW, Sun YX, et al. STAT5 promotes PD-L1 expression by facilitating histone lactylation to drive immunosuppression in acute myeloid leukemia. Signal Transduction and Targeted Therapy. 2023; 8: 391. https://doi.org/10.1038/s41392-023-01605-2

[22] Zeng Y, Huang Y, Tan Q, Peng L, Wang J, Tong F, et al. Influence of lactate in resistance to anti-PD-1/PD-L1 therapy: Mechanisms and clinical applications (Review). Molecular Medicine Reports. 2025; 31: 48. https://doi.org/10.3892/mmr.2024.13413

[23] Zhang D, Tang Z, Huang H, Zhou G, Cui C, Weng Y, et al. Metabolic regulation of gene expression by histone lactylation. Nature. 2019; 574: 575–580. https://doi.org/10.1038/s41586-019-1678-1

[24] Li F, Si W, Xia L, Yin D, Wei T, Tao M, et al. Positive feedback regulation between glycolysis and histone lactylation drives oncogenesis in pancreatic ductal adenocarcinoma. Molecular Cancer. 2024; 23: 90. https://doi.org/10.1186/s12943-024-02008-9

[25] Zang Y, Wang A, Zhang J, Xia M, Jiang Z, Jia B, et al. Hypoxia promotes histone H3K9 lactylation to enhance *LAMC2* transcription in esophageal squamous cell carcinoma. iscience. 2024; 27: 110188. https://doi.org/10.1016/j.isci.2024.110188

[26] Barba I, Carrillo-Bosch L, Seoane J. Targeting the Warburg Effect in Cancer: Where Do We Stand? International Journal of Molecular Sciences. 2024; 25: 3142. https://doi.org/10.3390/ijms25063142

[27] Alzial G, Renoult O, Paris F, Gratas C, Clavreul A, Pecqueur C. Wild-type isocitrate dehydrogenase under the spotlight in glioblastoma. Oncogene. 2022; 41: 613–621. https://doi.org/10.1038/s41388-021-02056-1

[28] Marcucci F, Rumio C. The Role of Oxidative Metabolism in Tumorigenesis and Drug Resistance. Discovery Medicine. 2024; 36: 1109–1126. https://doi.org/10.24976/Discov.Med.202436185.102

[29] Minami E, Sasa K, Yamada A, Kawai R, Yoshida H, Nakano H, et al. Lactate-induced histone lactylation by p300 promotes osteoblast differentiation. PloS One. 2023; 18: e0293676. https://doi.org/10.1371/journal.pone.0293676

[30] Lin X, Kang K, Chen P, Zeng Z, Li G, Xiong W, et al. Regulatory mechanisms of PD-1/PD-L1 in cancers. Molecular Cancer. 2024; 23: 108. https://doi.org/10.1186/s12943-024-02023-w

[31] Liu Z, Yu X, Xu L, Li Y, Zeng C. Current insight into the regulation of PD-L1 in cancer. Experimental Hematology & Oncology. 2022; 11: 44. https://doi.org/10.1186/s40164-022-00297-8

[32] Yamaguchi H, Hsu JM, Yang WH, Hung MC. Mechanisms regulating PD-L1 expression in cancers and associated opportunities for novel small-molecule therapeutics. Nature Reviews. Clinical Oncology. 2022; 19: 287–305. https://doi.org/10.1038/s41571-022-00601-9

[33] Dong M, Zhang Y, Chen M, Tan Y, Min J, He X, et al. ASF1A-dependent P300-mediated histone H3 lysine 18 lactylation promotes atherosclerosis by regulating EndMT. Acta Pharmaceutica Sinica. B. 2024; 14: 3027–3048. https://doi.org/10.1016/j.apsb.2024.03.008

[34] Soltani M, Abbaszadeh M, Fouladseresht H, Sullman MJM, Eskandari N. PD-L1 importance in malignancies comprehensive insights into the role of PD-L1 in malignancies: from molecular mechanisms to therapeutic opportunities. Clinical and Experimental Medicine. 2025; 25: 106. https://doi.org/10.1007/s10238-025-01641-y

[35] Schmid-Siegert E, Qin M, Tian H, Arpat B, Chen B, Xenarios I. Reference genomes for BALB/c Nude and NOD/SCID mouse models. G3 (Bethesda, Md.). 2023; 13: jkad188. https://doi.org/10.1093/g3journal/jkad188

[36] Kim EY, Abides J, Keller CR, Martinez SR, Li W. Tumor Microenvironment Lactate: Is It a Cancer Progression Marker, Immunosuppressant, and Therapeutic Target? Molecules (Basel, Switzerland). 2025; 30: 1763. https://doi.org/10.3390/molecules30081763