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Histone lactylation modulates apoptosis and stress response through PI3K/AKT signaling in GC-1 spermatogonial cells under hypoxia

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Abstract

Background: Histone lactylation is a recently identified epigenetic modification arising from lactate metabolism. This study aimed to determine whether lactate-induced histone lactylation contributes to the regulation of apoptosis in mouse Germ Cell-1 (GC-1) cells under hypoxic stress. **Methods**: A hypoxia-induced cell model was established to examine lactate accumulation, histone lactylation, and apoptosis in GC-1 cells. The experimental groups included the normoxia control (Con group), hypoxia (Hy group), hypoxia + sodium oxamate (Hy + Oxa group), and L-lactate treatment (Nala group). Lactate levels, histone lactylation, apoptosis-related gene and protein expression, and apoptosis rates were assessed by lactate assays, Western blot, immunofluorescence, quantitative real-time polymerase chain reaction (qRT-PCR), and Annexin V/propidium iodide (PI) flow cytometry. **Results**: After 36 h of hypoxia, cell viability decreased to (58.55% $\pm$ 6.20%) ($p < 0.001$). Hypoxia exposure caused increase in intracellular lactate concentration and induced histone lactylation ($p < 0.01$). Compared with the Con group, cells in the Hy group exhibited increased expression of pro-apoptotic markers Bax and caspase-3, reduced Bcl-2 expression, and higher apoptosis levels ($p < 0.05$). The Hy + Oxa group showed reduced histone lactylation and decreased apoptosis compared with the Hy group ($p < 0.05$). The Nala group exhibited patterns similar to the Hy group, including increased histone lactylation and enhanced apoptosis ($p < 0.05$), confirming that lactate regulates apoptosis through histone lactylation. Furthermore, both hypoxia and exogenous lactate significantly reduced the ratios of phosphorylated phosphatidylinositol 3-kinase (p-PI3K)/PI3K and phosphorylated protein kinase B (p-AKT)/AKT, whereas oxamate partially restored their activity ($p < 0.05$). **Conclusions**: Hypoxia-induced lactate accumulation promotes apoptosis of GC-1 cells by enhancing histone lactylation, particularly at the H3K18 site, partly through suppression of the PI3K/AKT signaling pathway. These findings provide new mechanistic insights into hypoxia-associated male infertility and suggest that histone lactylation may represent a potential therapeutic target.

Keywords

Histone lactylation; Hypoxia; Spermatogonia; Apoptosis; Lactate; GC-1 cells

La lactilación de histonas modula la apoptosis y la respuesta al estrés mediante la vía de señalización PI3K/AKT en células espermatogoniales GC-1 bajo hipoxia

Resumen

Antecedentes: La lactilación de histonas es una modificación epigenética que se deriva del metabolismo del lactato. Este estudio tuvo como objetivo determinar si la lactilación de histonas inducida por lactato contribuye a la regulación de la apoptosis en células espermatogoniales de ratón Germ Cell-1 (GC-1) bajo estrés hipóxico. **Métodos**: Se estableció un modelo celular inducido por hipoxia para examinar la acumulación de lactato, la lactilación de histonas y la apoptosis en células GC-1. Los grupos experimentales incluyeron: control en normoxia (grupo Con), hipoxia (grupo Hy), hipoxia + oxamato sódico (grupo Hy + Oxa) y tratamiento con L-lactato (grupo Nala). Los niveles de lactato, la lactilación de histonas, la expresión de genes y proteínas relacionados con la apoptosis y las tasas de apoptosis se evaluaron mediante ensayos de lactato, Western blot, inmunofluorescencia, reacción en cadena de la polimerasa cuantitativa a tiempo real (qRT-PCR) y citometría de flujo con Annexina V/yoduro de propidio (PI). **Resultados**: Después de 36 h de hipoxia, la viabilidad celular disminuyó a (58.55% $\pm$ 6.20%) ($p < 0.001$). La exposición a la hipoxia causó un aumento en la concentración intracelular de lactato e indujo lactilación de histonas ($p < 0.01$). En comparación con el grupo Con, las células del grupo Hy exhibieron un aumento en la expresión de los marcadores proapoptóticos Bax y caspasa-3, una expresión reducida de Bcl-2 y mayores niveles de apoptosis ($p < 0.05$). El grupo Hy + Oxa mostró una lactilación de histonas reducida y una disminución de la apoptosis en comparación con el grupo Hy ($p < 0.05$). El grupo Nala exhibió patrones similares al grupo Hy, incluido el aumento de la lactilación de histonas y la apoptosis ($p < 0.05$), lo que confirma que el lactato regula la apoptosis a través de la lactilación de histonas. Además, tanto la hipoxia como el lactato exógeno redujeron significativamente las razones de fosfatidilinositol 3-quinasa fosforilada (p-PI3K)/PI3K y proteína quinasa B fosforilada (p-AKT)/AKT, mientras que el oxamato restauró parcialmente su actividad ($p < 0.05$). **Conclusiones**: La acumulación de lactato inducida por hipoxia promueve la apoptosis de las células espermatogoniales GC-1 al potenciar la lactilación de histonas, particularmente en el sitio H3K18, parcialmente mediante la supresión de la vía de señalización PI3K/AKT. Estos hallazgos proporcionan nuevas perspectivas mecanísticas sobre la infertilidad masculina asociada a la hipoxia y sugieren que la lactilación de histonas puede representar una diana terapéutica potencial.

Palabras Clave

Lactilación de histonas; Hipoxia; Espermatogonias; Apoptosis; Lactato; Células GC-1

1. Introduction

Infertility, which affects nearly 15% of couples of reproductive age [1], is a prevalent reproductive health concern worldwide. A diagnosis of infertility is made when pregnancy does not occur after 12 months or more of regular, unprotected intercourse [2, 3]. Male factors contribute to approximately 40% of these cases [2, 4], and their etiology involves a complex interplay of genetic, environmental, and epigenetic regulatory mechanisms [5].

Under physiological conditions, regions of the seminiferous epithelium are naturally exposed to mild hypoxia (7%–13% O_2) because of limited vascularization. This level of hypoxia helps to maintain germ cell homeostasis, restrain excessive reactive oxygen species (ROS) accumulation, and sustain hypoxia-inducible factor (*HIF*) signaling, all of which are essential for normal spermatogenesis. By contrast, pathological hypoxia caused by conditions such as varicocele, testicular torsion, or systemic hypoxia results in excessive ROS generation, mitochondrial dysfunction, and activation of germ cell apoptotic pathways, ultimately impairing spermatogenesis and contributing to male infertility. Since impaired spermatogenesis is a primary cause of infertility and often manifests as decreased semen quality, dysfunction of the spermatogenic process is recognized as a fundamental mechanism that precedes and directly drives fertility decline in men [6, 7]. Spermatogenesis, the process by which sperm are produced, is indispensable not only for the transfer of genetic material but also for the perpetuation of species [6]. This process requires the coordinated function of spermatogonia, Sertoli cells, and Interstitial cells, all of which sustain sperm production [6]. However, exposure to hypoxic conditions can disrupt spermatogenesis and induce germ cell apoptosis by altering metabolism and cellular function, ultimately reducing sperm quality and sperm count [6].

Lactate, the end product of glycolysis, accumulates under hypoxic conditions and plays a central role in regulating stress responses and metabolic reprogramming [8]. *HIF* enhances glycolysis by upregulating enzymes such as lactate dehydrogenase A (LDHA) and through interaction with *p300*, promotes lactate accumulation, which in turn, acts as a substrate for histone lysine lactylation (Kla), thereby linking metabolism to epigenetic regulation [9].

Recent studies have demonstrated that lactate does more than act as a metabolic byproduct; it also induces epigenetic modifications by promoting histone lactylation [10–12]. This post-translational modification involves the covalent addition of lactate to lysine residues on histones, particularly on histone H3 (K9, K14, K18, and K23) and histone H4 (K5, K8, K12, and K16). By altering these sites, histone lactylation regulates chromatin structure and transcriptional activity, thereby modifying gene expression in response to elevated lactate

levels [10]. Increasing evidence indicates that histone lactylation contributes to the pathophysiology of diverse conditions, including cancer, cardiovascular and neurological diseases, respiratory dysfunction, and immune-related disorders [13].

Although global pan-lactylation studies suggest that multiple histone sites are associated with apoptosis regulation, recent investigations have consistently highlighted histone H3 lysine 18 lactylation (H3K18la) as a particularly important mediator of apoptotic regulation across diverse cellular contexts [14–16]. Histone lactylation affects cell proliferation, survival, and apoptosis [17], but its precise role appears to vary with disease model, cell type, and molecular background. In certain conditions, lactylation exerts anti-apoptotic effects. For instance, Lin *et al*. [18] showed that in myocardial ischemia–reperfusion injury, lactate accumulation promotes H3K18la via p300/CREB-binding protein (CBP), which enhances Bcl-2 expression while suppressing Bax, and lactylation of Caspase-3 at lysine 14 blocks its cleavage and activation, thereby reducing mitochondrial-mediated apoptosis. Similarly, in endometrial carcinoma, Kla enhances ubiquitin-specific protease 39 (USP39) expression via PI3K/AKT/HIF-1α signaling, supporting tumor survival and growth [19]. Deng *et al*. [20] further reported that histone H4 lysine 12 lactylation (H4K12la) upregulates glutamate-cysteine ligase catalytic subunit (GCLC), elevates glutathione synthesis, and inhibits ferroptosis, thereby increasing resistance in colorectal cancer stem cells. In contrast, pro-apoptotic effects of histone lactylation have been observed in several stress- or hypoxia-related contexts. Xu *et al*. [16] found that H3K18la activates the Ras homolog gene family member A/Rho-associated coiled-coil containing protein kinase/Ezrin (RhoA/ROCK/Ezrin) pathway, which upregulates Bax and downregulates Bcl-2, thereby facilitating apoptosis. Qiao *et al*. [21] demonstrated that lactylation of H3K18 and Ezrin enhances inflammatory and apoptotic responses via nuclear factor-kappa B (NF-κB) signaling in sepsis-induced renal injury. Zhang *et al*. [14] reported that LDHA-driven lactate accumulation during intracerebral hemorrhage increases H3K18la on the p53 promoter, upregulating apoptotic genes and aggravating neuronal apoptosis. In addition, Li *et al*. [22] showed that pyruvate kinase M2 (PKM2) dimerization in aortic vascular smooth muscle cells (VSMCs) induces lactate accumulation and histone lactylation, opening chromatin regions near Bax and Caspase-3 while suppressing Bcl-2 expression, ultimately promoting apoptosis in VSMCs. These findings are consistent with our results, which demonstrated that hypoxia-induced lactate accumulation markedly increased H3K18la and was accompanied by elevated expression of Bax and Caspase-3 together with reduced Bcl-2 expression. Collectively, this suggests that although histone lactylation can exert diverse effects on apoptosis, under hypoxic conditions, its predominant role is to promote mitochondrial-dependent apoptosis. On this basis, GC-1 cell represent a valuable model for investigating whether histone lactylation modulates spermatogonial apoptosis during hypoxia, an area that remains insufficiently understood.

We hypothesize that under pathological hypoxia, changes in lactate metabolism and the resulting Kla may no longer serve a physiological protective function but instead drive the activation of pro-apoptotic signaling cascades. This functional shift from protection to injury highlights the need to elucidate the mechanistic basis of histone lactylation in pathological hypoxic contexts. Supporting this hypothesis, our experiments revealed that lactate-driven histone lactylation was accompanied by reductions in the p-PI3K/PI3K and p-AKT/AKT ratios, indicating that Kla may regulate apoptosis at least in part through modulation of the PI3K/AKT signaling pathway. To address this, the present study established a hypoxic GC-1 cell model and, by applying lactate supplementation and Kla inhibition assays, systematically investigated the molecular mechanisms through which the lactate—Kla axis promotes spermatogonial apoptosis under pathological hypoxia. Particular attention was given to its regulatory interaction with the PI3K/AKT pathway and its implications for spermatogenesis. We anticipate that these findings will enhance understanding of the molecular mechanisms underlying hypoxia-induced stress responses in the male reproductive system and provide a theoretical basis for the development of preventive and therapeutic strategies for related disorders.

2. Materials and methods

2.1 GC-1 cell culture and grouping

In this study, the mouse GC-1 cell was obtained from Shanghai Yuchun Biological Technology Co., Ltd. The cell line originates from the testis of a 10-day-old male mouse, immortalized by transfection with the pSV3-neo plasmid, with the catalog number CM0074. The cell line was authenticated using Short Tandem Repeat (STR) profiling and confirmed to be free of mycoplasma contamination. We also consulted the International Cell Line Authentication Committee (ICLAC) and ExPASy Cellosaurus databases to rule out any known misidentification or cross-contamination issues. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; L110KJ, Shanghai, China) supplemented with 10% fetal bovine serum (FBS; FBS500-S, Brisbane, QLD, Australia) and 1% penicillin-streptomycin solution (PB180120, Wuhan, China). The cultures were maintained in an incubator at 37 °C with 5% CO_2, the cells were passaged according to confluency and growth status, and when confluence reached 80%–90%, they were subcultured and adjusted to a density of 1.5×10^5 cells/mL for subsequent experiments for optimal viability and uniformity across experimental replicates. After 24 h of culture, the cells were divided into four experimental groups: Control group (Con): Cells were cultured under normoxic conditions (37 °C, 5% CO_2, 21% O_2) for 36 h. Hypoxia group (Hy): Cells were cultured in a hypoxic incubator (Thermo Fisher Scientific, Waltham, MA, USA; 37 °C, 3% O_2, 5% CO_2, 92% N_2) for 36 h to simulate hypoxia. Hypoxia + Sodium Oxamate group (Hy + Oxa): Cells were exposed to 20 mM sodium oxamate (CAS: 547-35-7; Sigma-Aldrich, St. Louis, MO, USA) under hypoxic conditions for 36 h. Sodium oxamate is a well-established LDHA inhibitor commonly used to suppress lactate production and histone lactylation under metabolic stress. The concentration of 20 mM was selected based on previous studies demonstrating its effectiveness in inhibiting LDHA activity and histone lactylation [23–27]. L-

lactate treatment group (Nala): Cells were treated with 20 mM sodium L-lactate (CAS: 72-17-3; Sigma-Aldrich, St. Louis, MO, USA) for 36 h to mimic lactate accumulation observed during hypoxic stress. This concentration has been widely used in previous studies to replicate pathological lactate levels, which typically range from 15–25 mM in severely hypoxic tissues or during intense metabolic activity [10–12, 28–30].

2.2 Lactate measurement in GC-1 cells

To assess lactate levels in the culture supernatants of the Con, Hy, and Hy + Oxa groups, cell culture media were collected following treatment. L-lactate concentrations were then quantified using a commercial assay kit (BC2235; Solarbio Science & Technology Co., Ltd., Beijing, China), following the manufacturer's instructions.

2.3 GC-1 cell viability assessment

GC-1 cells in the logarithmic growth phase were seeded into 96-well plates at a density of 5×10^3 cells per well. Cells were exposed to hypoxic conditions for 24, 30, 36, and 48 h. At each time point, the medium was removed, and wells were washed twice with phosphate-buffered saline (PBS). Subsequently, 100 μL of complete DMEM medium and 10 μL of Cell Counting Kit-8 (CCK-8) solution were added to each well. Plates were gently agitated and incubated at 37 °C for 1.5 h, after which absorbance was measured at 450 nm. Cell viability was calculated using the following formula: $R = [(A2 - Ao)/(A1 - Ao)] \times 100\%$, where Ao represents the absorbance of wells containing only medium and CCK-8 solution, A1 represents the absorbance of wells containing medium, cells, and CCK-8 solution under normoxic conditions, and A2 represents the absorbance of wells under hypoxic conditions. This calculation allowed direct comparison of cell viability between normoxic and hypoxic conditions.

2.4 Detection of histone lactylation, apoptosis markers, and PI3K/AKT pathway in GC-1 cells

Western blot analysis was performed on GC-1 cells from each experimental group. Cells were lysed in buffer containing 50 mmol/L Tris, 500 mmol/L sodium chloride, 10% glycerol, and 20 mmol/L imidazole (pH 8.0). After centrifugation, total protein was extracted from the cell lysates using the radioimmunoprecipitation assay (RIPA) method. Protein concentrations were determined using the bicinchoninic acid (BCA) assay, and samples were heat-denatured at 95 °C for 5 minutes before electrophoresis to ensure protein unfolding. Equal amounts of protein were separated by 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred onto polyvinylidene fluoride (PVDF) membranes, and blocked [6, 24]. The membranes incubated overnight at 4 °C with the following primary antibodies: anti-histone H3K18 lactylation (H3K18la) (1:800; PTM-1406; PTM Bio, Hangzhou, Zhejiang, China), anti-pan lysine lactylation (Pan-Kla) (1:500; PTM-1401; PTM Bio, Hangzhou, Zhejiang, China), anti-histone H3 (1:2000, BS-0349R, Abbkine, Beijing, China), anti-Bax (1:1000; 5023S; CST, Danvers, MA, USA), anti-Bcl-2 (1:1000; NB100-92142; NOVUS, Centennial, CO, USA), anti-Caspase-3 (1:1000; 9662S; CST, Danvers, MA, USA), anti-PI3K (1:1000; 4255S; CST, Danvers, MA, USA), p-PI3K (1:1000; 4228S; CST, Danvers, MA, USA), anti-AKT (1:1000; 4685S; CST, Danvers, MA, USA), and p-AKT (1:1000; 4060S; CST, Danvers, MA, USA). After three washes with Tris-buffered saline with Tween 20 (TBST; 5 min each), the membranes were incubated with secondary antibodies (1:3000) at room temperature for 1 hour. Protein bands were visualized using enhanced chemiluminescence (ECL) reagents and imaged on a chemiluminescence detection system [31]. Relative protein expression was quantified using ImageJ software (version 1.54k; National Institutes of Health, Bethesda, MD, USA). H3K18la and Pan-Kla were normalized to total H3; Bax, Bcl-2, and Caspase-3 were normalized to β-actin; and PI3K, p-PI3K, AKT, and p-AKT were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH), ensuring consistency across loading controls.

2.5 Quantification and localization of histone lactylation in GC-1 cells

The cells were seeded on slides, fixed, and permeabilized with 0.1% Triton X-100 for 10 minutes, followed by blocking with 5% goat serum for 1 hour [24]. The slides were then incubated overnight at 4 °C with primary antibodies: anti-Kla (1:500, 1401, PTM-1401; PTM Bio, Hangzhou, Zhejiang, China) and anti-H3K18la (1:800, PTM-1406; PTM Bio, Hangzhou, Zhejiang, China). After washing, samples were incubated for 1.5 h with Alexa Fluor 488-conjugated secondary antibodies (1:1000; BMD00063, Abcam, Cambridge, MA, USA). Nuclear staining was performed using 4′,6-diamidino-2-phenylindole (DAPI; BMD00063, Abbkine, Wuhan, Hubei, China) for 15 minutes. Fluorescence images were obtained using a ZEISS Axio Observer Z1 inverted fluorescence microscope (Carl Zeiss AG, Jena, TH, Germany). This allowed for the localization and visualization of histone lactylation within GC-1 cells, as well as the identification of relevant cellular structures.

2.6 Quantitative analysis of apoptosis marker gene expression in GC-1 cells

Total RNA was extracted using Trizol reagent, and cDNA was synthesized with a reverse transcription kit (3351285, Thermo Fisher Scientific, Waltham, MA, USA). Quantitative PCR was carried out using SYBR Green I fluorescent dye (SYBR) PCR Master Mix and gene-specific primers targeting apoptosis-related genes (Bax, Bcl-2, and Caspase-3). Amplification was performed on an Applied Biosystems (ABI) 7500 real-time PCR system. Gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method, with GAPDH serving as the internal reference. Primer sequences are provided in Table 1.

2.7 Apoptosis rate detection in GC-1 cells

Apoptosis was assessed using Annexin V/PI double staining according to the manufacturer's instructions (Annexin V/PI Detection Kit, Invitrogen, Catalog No. A13201; Invitrogen,

TABLE 1. Primer sequences for target genes.

Gene	Gene Sequence	bp
Bax-F1	GAACTGGACAGCAATATGGA	
Bax-R1	GAAGTTGCCATCAGCAAAC	111 bp
Bcl-2-F1	AGCCTTGGCCAGGGAATTAT	
Bcl-2-R1	GGACTTGGTGCATGGAACAC	160 bp
Caspase-3-F1	GTTCATCCAGTCCCTTTGCA	
Caspase-3-R1	CACGGGATCTGTTTCTTTGC	150 bp
β-actin-F1	GCTTCTAGGCGGACTGTTAC	
β-actin-R1	CCATGCCAATGTTGTCTCTT	100 bp

Bax, Bcl-2-associated X protein; Bcl-2, B-cell lymphoma 2; Caspase-3, cysteine-dependent aspartate-specific protease 3; β-actin, beta-actin; bp, base pairs.

Waltham, MA, USA). After washing the cells with PBS, Annexin V-fluorescein isothiocyanate (V-FITC) and propidium iodide (PI) solutions were added, and the samples were incubated at room temperature for 15 minutes. The apoptosis rate was then analyzed using a BD FACSCanto II flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA), and data were processed with FlowJo software (version 10.8.1; FlowJo LLC, Ashland, OR, USA). The proportions of early apoptotic, late apoptotic, and live cells were calculated to evaluate the impact of histone lactylation on apoptosis in hypoxic spermatogonia.

2.8 Statistical analysis

All experiments were performed with at least three biological replicates. Data are presented as mean ± standard deviation ($\bar{x} \pm s$). Statistical analyses were performed using SPSS 27.0 (IBM Corp., Armonk, NY, USA), and graphs were generated with GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, USA). For comparisons between two groups, independent-sample *t*-tests were used, while one-way analysis of variance (ANOVA) was applied for comparisons among multiple groups. A p-value < 0.05 was considered statistically significant.

3. Results

3.1 Effects of different hypoxic durations on GC-1 cells

3.1.1 Hypoxia-induced apoptosis in GC-1 cells

To investigate the time-dependent effects of hypoxia on apoptosis, the expression levels of Bax, Bcl-2, and Caspase-3 were examined in GC-1 cells exposed to normoxia or hypoxia for 24, 30, 36, or 48 h. Compared with the normoxia group, expression of the pro-apoptotic gene Bax significantly increased with prolonged hypoxia ($p < 0.01$), whereas expression of the anti-apoptotic gene Bcl-2 gradually decreased ($p < 0.05$). Expression of Caspase-3, an executioner caspase activated in the terminal phase of apoptosis, also progressively increased ($p < 0.05$). These results demonstrate that hypoxic stress promotes the activation of pro-apoptotic pathways and suppresses anti-apoptotic mechanisms in a time-dependent manner. Such findings highlight the progressive effect of hypoxia on spermatogonial apoptosis and provide a basis for exploring the potential regulatory contribution of histone lactylation in this process (Fig. 1).

3.1.2 Effects of different hypoxic durations on GC-1 cell viability

Cell viability analysis showed that, compared with the Con group, no significant difference was observed after 24 h of hypoxia ($p > 0.05$). However, by 30 h, viability began to decline ($p < 0.05$). After 36 h of hypoxia, viability decreased markedly to (58.55% ± 6.20%) ($p < 0.01$), a level considered appropriate for subsequent experiments. At 48 h, cell damage was more pronounced, with viability further reduced ($p < 0.05$), apoptotic gene expression (Bax, Caspase-3, and Bcl-2) was highly imbalanced, suggesting that the cells had entered late-stage apoptosis, a condition in which regulatory pathways may no longer accurately reflect physiological processes. Based on these findings, 36 h of hypoxia was selected as the optimal experimental time point, as it provided a balance between measurable injury and mechanistic relevance (Fig. 2).

3.2 Lactate levels in GC-1 cells under hypoxic conditions

To evaluate the impact of hypoxia on lactate accumulation in GC-1 cells, lactate concentrations were measured in the Con and Hy groups using a lactate detection kit. Lactate levels in the Hy group were 1.9-fold higher than those in the Con group ($p < 0.001$), indicating that hypoxia markedly promotes lactate accumulation. This increase is likely attributable to enhanced anaerobic glycolysis, representing a metabolic adaptation to oxygen deprivation. Furthermore, the addition of the lactate dehydrogenase inhibitor sodium oxamate (Hy + Oxa group) during hypoxic culture reduced lactate levels by 40.7% compared with the Hy group ($p < 0.01$), demonstrating that sodium oxamate effectively attenuated hypoxia-induced lactate accumulation (Fig. 3).

3.3 Effects of hypoxia on histone lactylation levels in GC-1 cells

Histone lactylation modifications were assessed by Western blot analysis. Compared with the Con group, the Hy group

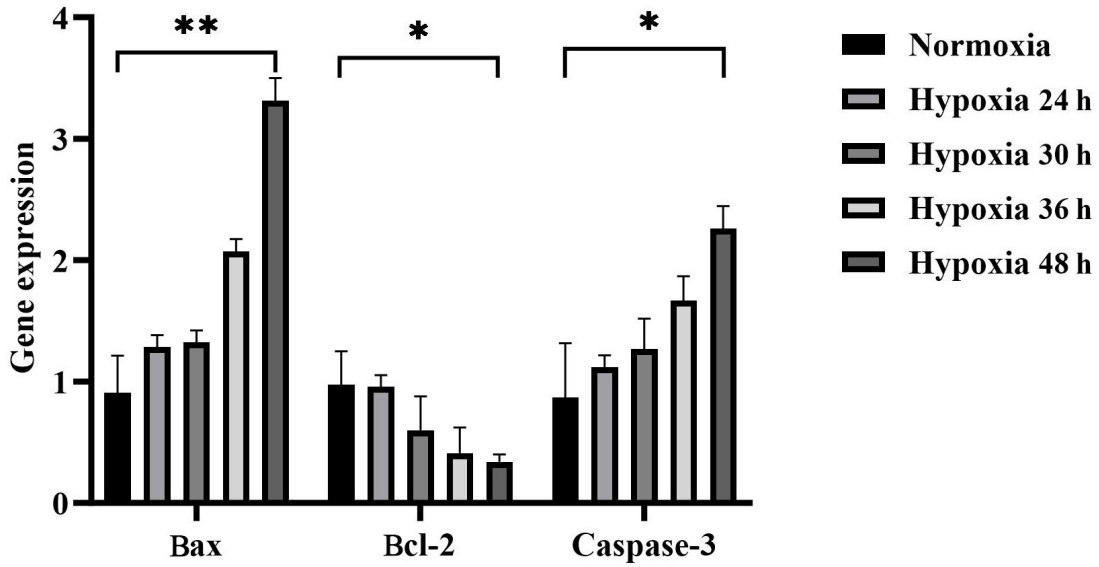


FIGURE 1. mRNA expression levels of apoptosis-related genes (*Bax*, *Bcl-2*, and *Caspase-3*) in GC-1 cells under different hypoxic treatments. GC-1 cells were exposed to hypoxia for 24, 30, 36, or 48 h. Quantitative real-time PCR was performed to measure the expression of the pro-apoptotic genes *Bax* and *Caspase-3*, and the anti-apoptotic gene *Bcl-2*, with normalization to β-actin. Data are presented as mean ± SD (n = 3). Hypoxia significantly upregulated *Bax* and *Caspase-3* (*$p < 0.05$), while downregulating *Bcl-2*, compared with the control group. *Bax*, Bcl-2-associated X protein; *Bcl-2*, B-cell lymphoma 2; *Caspase-3*, cysteine-dependent aspartate-specific protease 3. *$p < 0.05$, **$p < 0.01$.

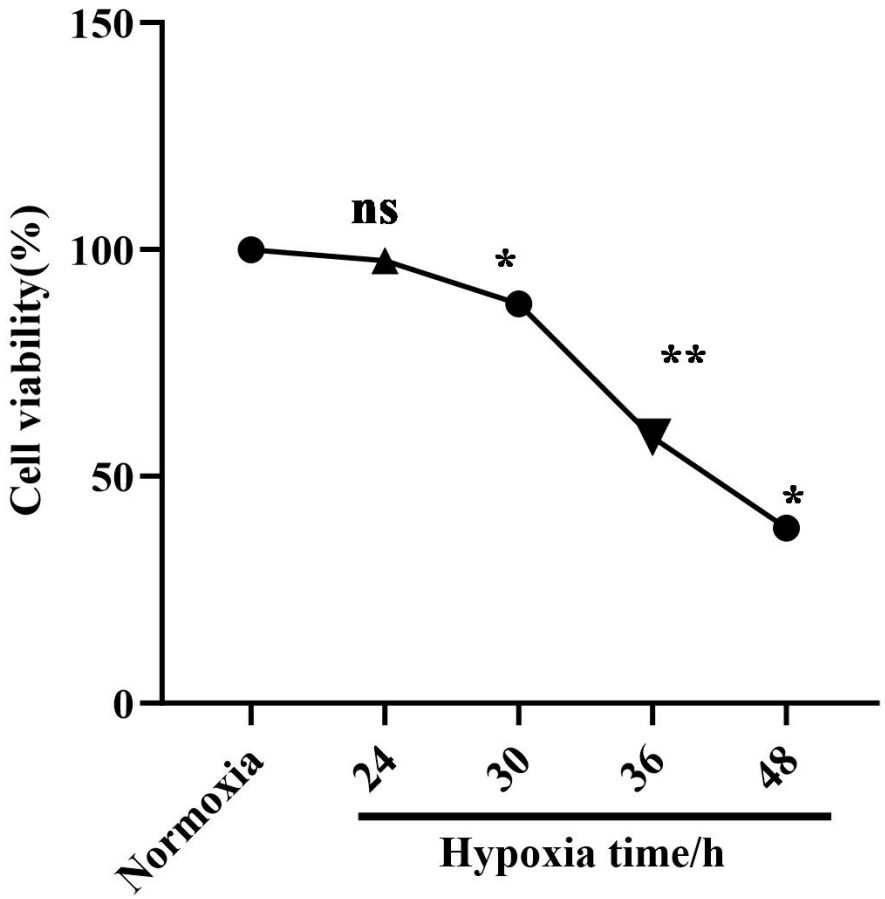
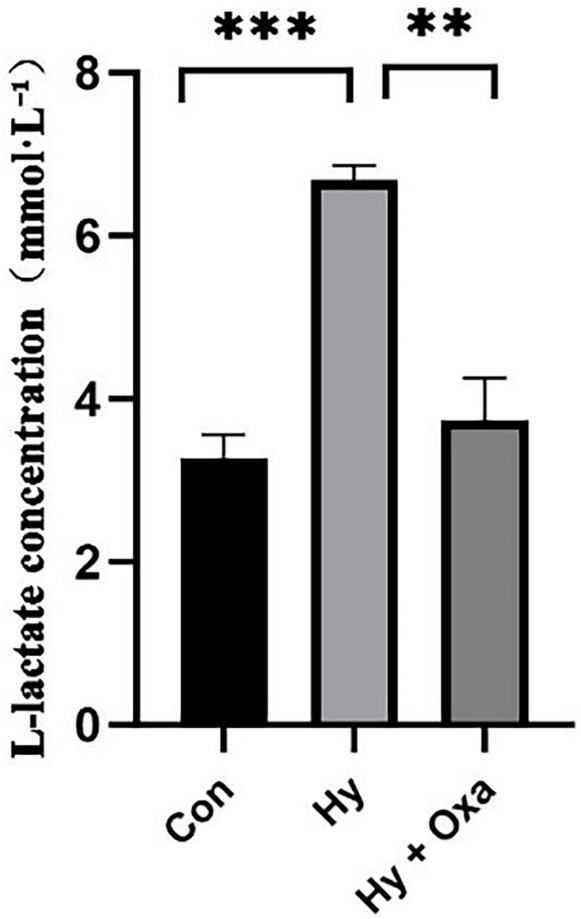


FIGURE 2. Cell viability of GC-1 cells under different durations of hypoxia. Data are presented as mean ± SD (n = 3). GC-1 cell viability was assessed after exposure to hypoxia for 24, 30, 36, and 48 h. Significant decreases were observed at 30 h, 36 h, and 48 h compared with the previous time points, whereas no significant difference was detected between normoxia and 24 h. ns, not significant; *$p < 0.05$; **$p < 0.01$.



F I G U R E 3. Lactate levels in GC-1 cells under different conditions. Data are presented as mean ± SD (n = 3). The Hy group exhibited significantly higher lactate levels compared with the Con group. Addition of the lactate dehydrogenase inhibitor sodium oxamate (Hy + Oxa group) during hypoxic culture reduced lactate levels relative to Hy. Con, normoxia group; Hy, hypoxia group; Hy + Oxa, hypoxia + sodium oxamate. $^{**}p < 0.01$, $^{***}p < 0.001$.

showed a pronounced increase in both pan-lactylation (Pan-Kla) and histone H3K18 lactylation (H3K18la). Specifically, H3K18la expression in the Hy group was elevated by approximately 12-fold relative to the Con group ($p < 0.001$), while Pan-Kla levels were increased by about 78% ($p < 0.001$). These findings indicate that hypoxia strongly activates histone lactylation, likely due to lactate accumulation driven by anaerobic glycolysis. Such modifications may directly or indirectly promote histone lactylation, thereby influencing epigenetic regulation and contributing to the cellular response to hypoxic stress (Fig. 4).

3.4 Effects of regulating lactate levels on histone lactylation in GC-1 cells

To further assess the role of lactate accumulation in histone lactylation, the expression of Pan-Kla and H3K18la was examined by Western blotting and immunofluorescence. Compared with the Con group, the Hy group displayed significantly elevated histone lactylation, with H3K18la increased by approximately 2.2-fold ($p < 0.05$) and Pan-Kla by 2.2-fold ($p < 0.001$). In contrast, the Hy + Oxa group exhibited markedly reduced histone lactylation, with H3K18la decreased by ∼74% and Pan-Kla by ∼66% relative to the Hy group, demonstrating that sodium oxamate effectively inhibited lactate production and suppressed histone lactylation. These findings underscore the pivotal role of lactate in modulating histone lactylation. Conversely, supplementation with sodium L-lactate (Nala) under normoxic conditions significantly enhanced histone lactylation, with H3K18la elevated by ∼2.8-fold and Pan-Kla increased by ∼33% compared with the Con group, which indicates that exogenous lactate could be sufficient to induce histone lactylation even in the absence of hypoxia. Together, these results demonstrate that lactate directly drives histone lactylation and that manipulating lactate levels can alter the extent of histone lactylation modifications in GC-1 cells (Fig. 5).

Immunofluorescence staining further corroborated these findings. Under hypoxic conditions, GC-1 cells displayed markedly enhanced fluorescence signals for H3K18la (Fig. 6) and Pan-Kla (Fig. 7), with the signals predominantly localized within the nucleus. This distribution indicates that histone lactylation modifications occur mainly in the nuclear compartment, where they are likely to regulate the transcription and expression of hypoxia-related genes, thereby influencing cellular physiological functions. These results suggest that histone lactylation represents an important adaptive mechanism through which cells respond to hypoxic environments.

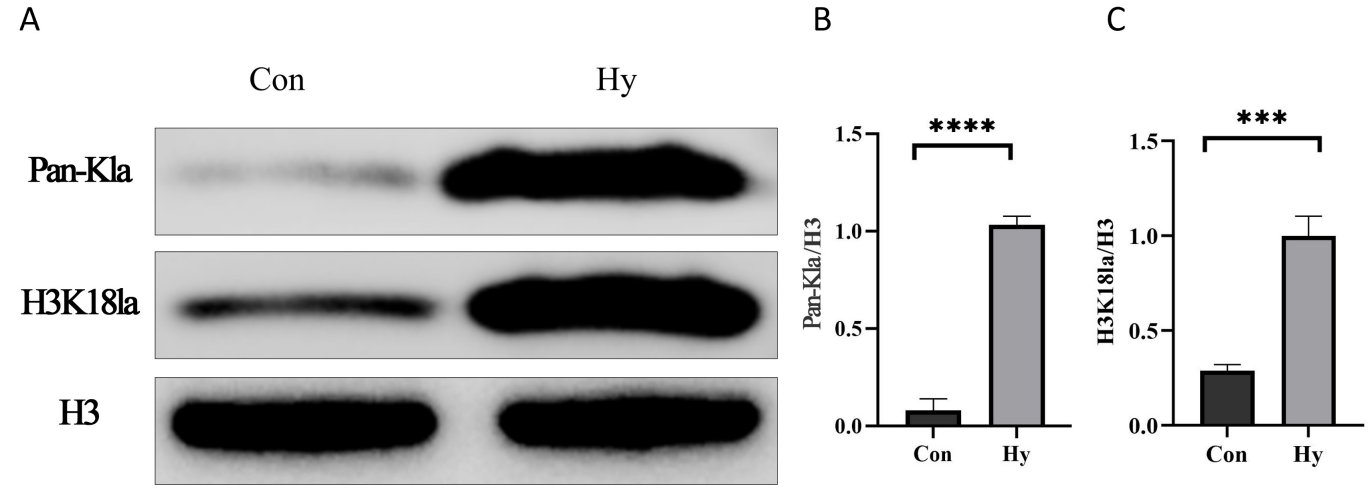


FIGURE 4. Western blot analysis of histone lactylation in GC-1 cells under normoxia and hypoxia. Data are presented as mean ± SD (n = 3). (A) western blot images showing protein levels of H3K18la and Pan-Kla. (B) Pan-Kla protein expression levels. (C) H3K18la protein expression levels. Hypoxia significantly increased global histone lactylation (Pan-Kla) and H3K18la compared with normoxia, indicating enhanced lactylation in response to low oxygen tension. ***$p < 0.001$; ****$p < 0.0001$. Con, normoxia group; Hy, hypoxia group; H3K18la, histone H3 lysine 18 lactylation; Pan-Kla, pan lysine lactylation.

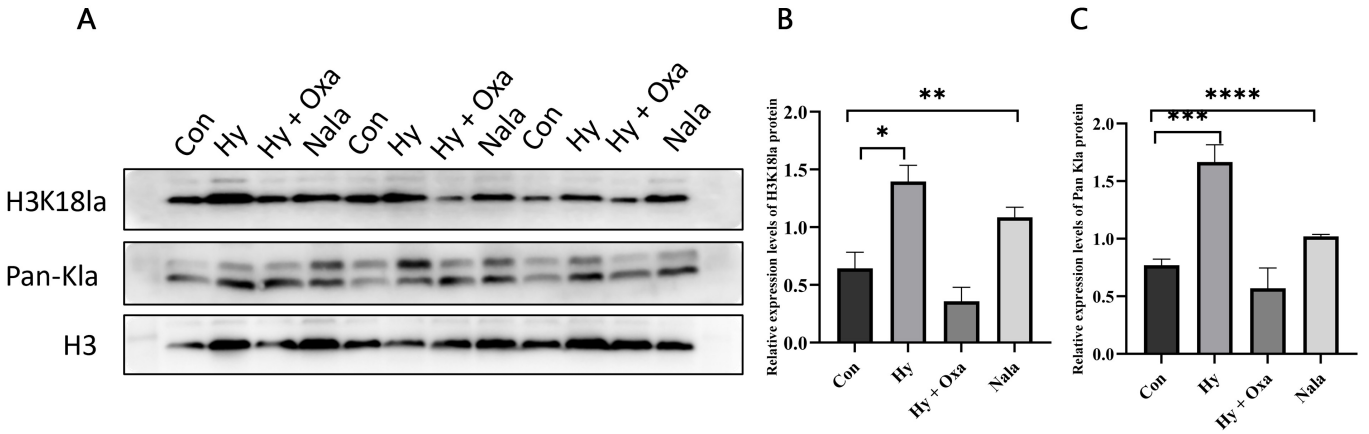


FIGURE 5. WB analysis of Pan-Kla and H3K18la levels in GC-1 cells under different treatments. Data are presented as mean ± SD (n = 3). (A) western blot images showing protein levels of H3K18la and Pan-Kla. (B) H3K18la protein expression levels. (C) Pan-Kla protein expression levels. The Con group showed low expression, whereas the Hy group exhibited a marked increase in Pan-Kla and H3K18la. Treatment with sodium oxamate (Hy + Oxa group) significantly reduced lactylation levels compared with Hy, while exogenous lactate (Nala) restored levels comparable to Hy. Con, normoxia group; Hy, hypoxia group; Hy + Oxa, hypoxia + sodium oxamate; Nala, L-lactate treatment; H3K18la, histone H3 lysine 18 lactylation; Pan-Kla, pan lysine lactylation. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

3.5 Lactate-regulated histone lactylation promotes apoptosis in GC-1 cells under hypoxic conditions

3.5.1 Protein expression of apoptosis markers

To determine how lactate-regulated histone lactylation influences apoptosis, we analyzed the protein expression of Bax, Bcl-2, and Caspase-3. Compared with the Con group, the Hy group exhibited significant upregulation of Bax ($p < 0.01$) and Caspase-3 ($p < 0.05$), accompanied by a marked reduction in Bcl-2 expression ($p < 0.01$). These findings confirm that hypoxia promotes apoptotic signaling in GC-1 cells. In the Hy + Oxa group, Bax and Caspase-3 expression levels were reduced, while Bcl-2 expression was partially restored relative to the Hy group ($p < 0.05$), suggesting that inhibition of histone lactylation attenuates hypoxia-induced apoptosis. Conversely, the Nala group showed a protein expression profile similar to that of the Hy group, with increased Bax and Caspase-3 levels and decreased Bcl-2 expression compared with the Con group ($p < 0.05$). These results indicate that lactate-driven histone lactylation modulates apoptosis-related proteins in GC-1 cells under hypoxic stress (Fig. 8).

3.5.2 mRNA expression levels of apoptosis markers

To further elucidate the role of histone lactylation in GC-1 cell apoptosis, quantitative PCR was used to measure the mRNA expression of *Bax*, *Bcl-2*, and *Caspase-3* across the different treatment groups. ANOVA revealed significant differences

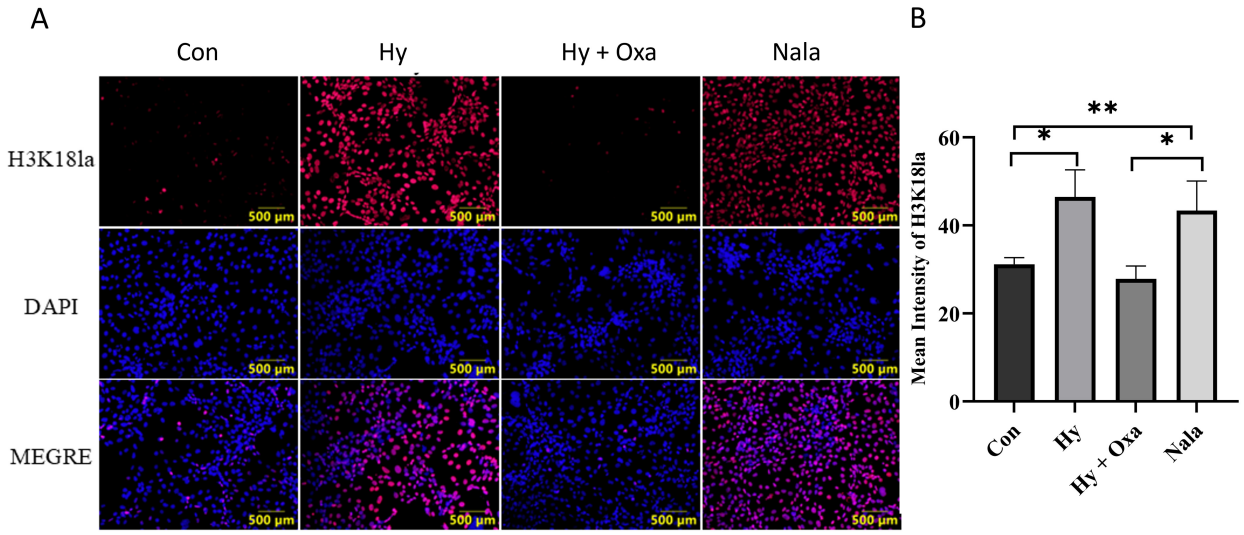


FIGURE 6. Immunofluorescence analysis of H3K18la expression and quantification in GC-1 cells under different treatments. (A) Representative immunofluorescence images showing H3K18la expression with DAPI nuclear staining. Scale bar: 500 μm. (B) Quantitative analysis of H3K18la fluorescence intensity. Data are presented as mean ± SD (n = 3). Fluorescence intensity was significantly increased in the Hy group compared with Con, suppressed by sodium oxamate (*$p < 0.05$), and elevated again by exogenous lactate relative to Con. *$p < 0.05$; **$p < 0.01$. Con, normoxia group; Hy, hypoxia group; Hy + Oxa, hypoxia + sodium oxamate; Nala, L-lactate treatment; H3K18la, histone H3 lysine 18 lactylation; DAPI, 4′,6-diamidino-2-phenylindole; MERGE, merged images.

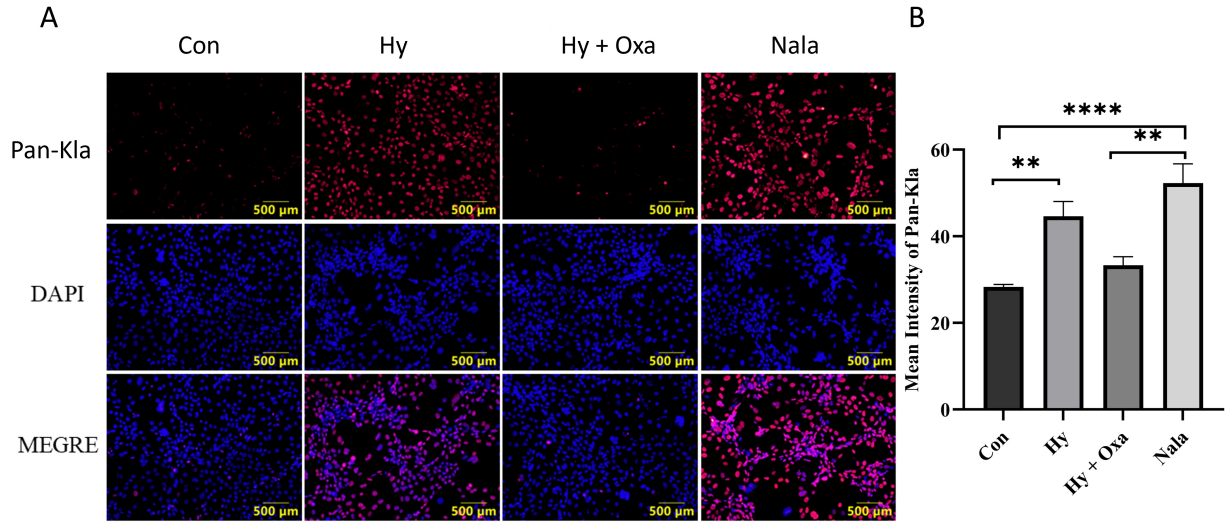


FIGURE 7. Immunofluorescence analysis of Pan-Kla expression and quantification in GC-1 cells under different treatments. (A) Representative immunofluorescence images showing Pan-Kla expression with DAPI nuclear staining and merged images. Scale bar: 500 μm. (B) Quantitative analysis of Pan-Kla fluorescence intensity. Data are presented as mean ± SD (n = 3). Compared with Con, Pan-Kla fluorescence intensity was significantly elevated in the Hy group and reduced in the Hy + Oxa group compared with Hy. Nala treatment induced higher Pan-Kla expression than Hy + Oxa. Con, normoxia group; Hy, hypoxia group; Hy + Oxa, hypoxia + sodium oxamate; Nala, normoxia + L-lactate; Nala, L-lactate treatment; Pan-Kla, pan lysine lactylation; DAPI, 4′,6-diamidino-2-phenylindole; MERGE, merged images. **$p < 0.01$; ****$p < 0.0001$.

among groups ($p < 0.05$). In the Hy group, *Bax* mRNA expression was 2.3-fold higher than in the Con group, *Caspase-3* increased by 73%, and *Bcl-2* decreased by 44%. In the Hy + Oxa group, *Bax* and *Caspase-3* expression levels were reduced by 44% and 56%, respectively, compared with the Hy group, while *Bcl-2* expression increased by 1.3-fold. These results suggest that inhibition of histone lactylation alleviates cellular stress and reduces apoptotic signaling. By contrast, the Nala group displayed a profile similar to the Hy group, with *Bax* expression elevated 4.6-fold relative to Con, *Caspase-3* increased 2.35-fold, and *Bcl-2* reduced by 61%. Together, these findings indicate that lactate-driven histone lactylation regulates apoptosis-related gene expression, thereby amplifying stress responses and promoting programmed cell death in GC-1 cells under hypoxic conditions (Fig. 9).

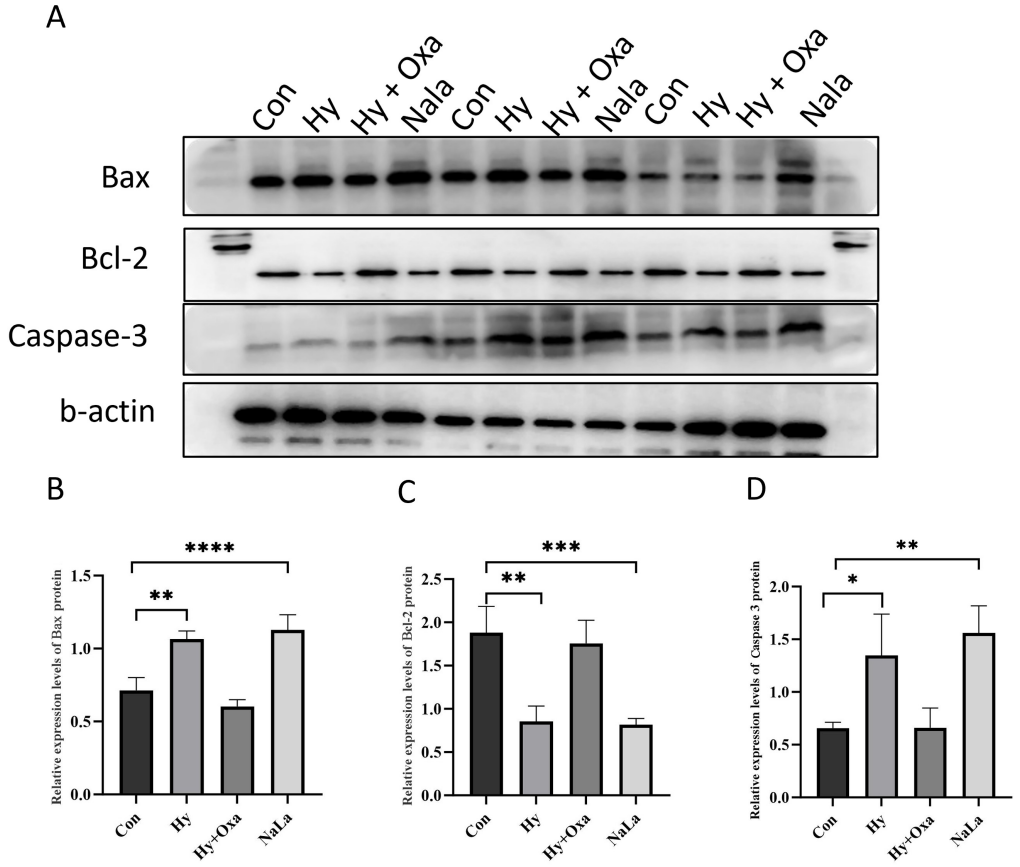


FIGURE 8. Western blot analysis of apoptosis-related proteins in GC-1 cells under different treatments. (A) Representative Western blot images of Bax, Bcl-2, and Caspase-3 protein expression. (B–D) Quantitative analysis of (B) Bax, (C) Bcl-2, and (D) Caspase-3 protein expression levels normalized to β-actin. Data are presented as mean ± SD (n = 3). In the Hy group, Bax and Caspase-3 expression were significantly upregulated, while Bcl-2 expression was significantly downregulated compared with Con. These changes were reversed in the Hy + Oxa group. Con, normoxia group; Hy, hypoxia group; Hy + Oxa, hypoxia + sodium oxamate; Nala, L-lactate treatment; Bax, Bcl-2-associated X protein; Bcl-2, B-cell lymphoma 2; Caspase-3, cysteine-dependent aspartate-specific protease 3. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

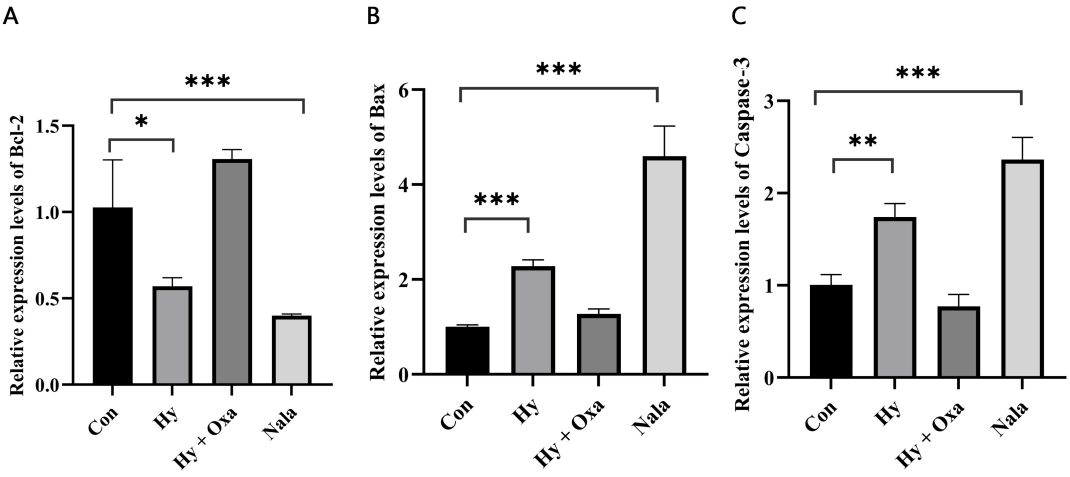


FIGURE 9. qRT-PCR analysis of apoptosis-related gene expression in GC-1 cells. Data are presented as mean ± SD (n = 3). (A) Bax, (B) Bcl-2, and (C) Caspase-3 relative expression levels. Expression levels of Bcl-2, Bax, and Caspase-3 were quantified by qRT-PCR. In the Hy group, Bcl-2 expression was significantly decreased (*$p < 0.05$), while Bax and Caspase-3 were significantly increased (***$p < 0.001$ and **$p < 0.01$, respectively) compared with Con. Treatment with sodium oxamate (Hy + Oxa) significantly reversed all three changes relative to Hy (***$p < 0.001$). *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. Con, normoxia group; Hy, hypoxia group; Hy + Oxa, hypoxia + sodium oxamate; Nala, L-lactate treatment; *Bax*, Bcl-2-associated X protein; *Bcl-2*, B-cell lymphoma 2; *Caspase-3*, cysteine-dependent aspartate-specific protease 3.

3.5.3 Apoptosis rate detection in GC-1 cells

Flow cytometric analysis showed that the apoptosis rate in the Hy group was significantly elevated compared with the Con group, with both early and late apoptotic populations increased. Specifically, early apoptosis increased 1.9-fold and late apoptosis increased 2.1-fold ($p < 0.01$). These findings are consistent with the Western blot and PCR results, confirming that hypoxia activates pro-apoptotic signaling pathways and promotes apoptosis in GC-1 cells. In the Hy + Oxa group, apoptosis was significantly reduced relative to the Hy group ($p < 0.01$), with early apoptosis decreased by 37% and late apoptosis by 34%. This indicates that lactate-induced histone lactylation facilitates hypoxia-induced apoptosis, and its inhibition can mitigate this effect. By contrast, the Nala group displayed apoptosis rates comparable to those of the Hy group, with both early and late apoptosis significantly increased relative to Con ($p < 0.01$). Early apoptosis was ~3.5-fold higher than Con, and late apoptosis was ~3.4-fold higher. These results further demonstrate that lactate accumulation drives histone lactylation modifications, which in turn activate apoptosis in GC-1 spermatogonia (Fig. 10).

3.5.4 Expression levels of PI3K/AKT pathway proteins

To further investigate the signaling mechanisms underlying histone lactylation-induced apoptosis, we analyzed the PI3K/AKT pathway by examining the ratios of phosphorylated to total protein (p-AKT/AKT and p-PI3K/PI3K) in GC-1 cells subjected to different treatments (Fig. 11A). For p-AKT/AKT (Fig. 11B), the Con group exhibited a significantly higher ratio than the Hy group, with an approximately 1.6-fold increase ($p < 0.05$). Treatment with oxamate partially restored pathway activity, as the Hy + Oxa group showed a ~1.5-fold increase compared with Hy ($p < 0.01$). Conversely, Nala treatment under normoxic conditions markedly reduced the ratio, being 49% lower than Con ($p < 0.001$). For p-PI3K/PI3K (Fig. 11C), a similar trend was observed. The Con group was significantly higher than the Hy group, with a 1.4-fold increase ($p < 0.01$). Oxamate again exerted a corrective effect, as Hy + Oxa was ~1.2-fold higher than Hy ($p < 0.05$). In contrast, Nala further suppressed the ratio, showing a 38% reduction compared with Con ($p < 0.001$). Taken together, these findings demonstrate that hypoxia markedly suppresses PI3K/AKT signaling relative to normoxia, while

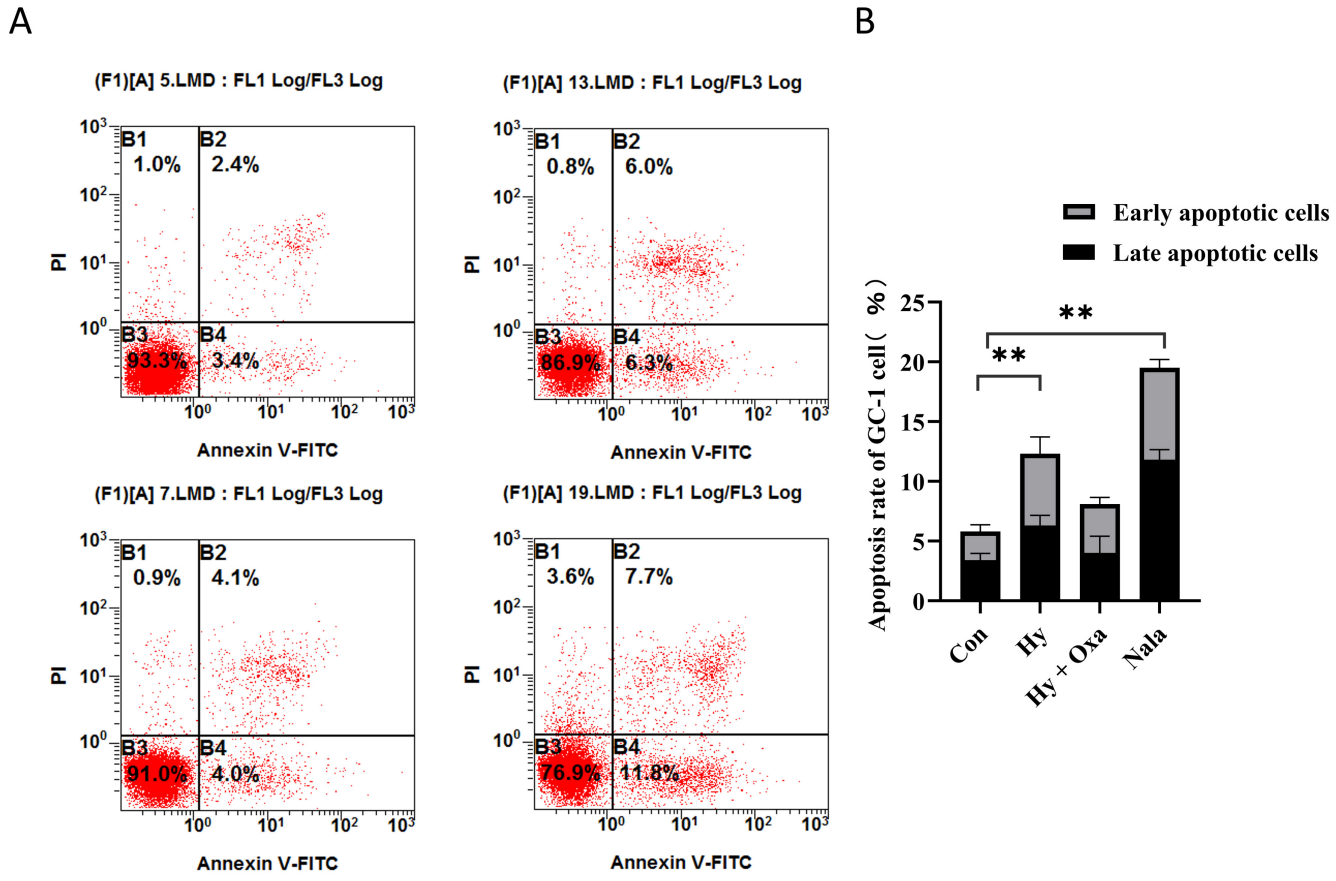


FIGURE 10. Apoptosis rate of GC-1 cells detected by flow cytometry. Data are presented as mean ± SD (n = 3). (A) Representative flow cytometry plots of Annexin V-FITC/PI double staining showing apoptotic cell populations in GC-1 cells under different treatments. (B) Statistical analysis of the total apoptosis rate. Total apoptosis (early + late) was significantly higher in the Hy group compared with Con. Treatment with sodium oxamate (Hy + Oxa) reduced apoptosis, whereas exogenous lactate (Nala) restored the rate to levels comparable to Hy. Statistical significance: $^{**}p < 0.01$. Con, normoxia group; Hy, hypoxia group; Hy + Oxa, hypoxia + sodium oxamate; Nala, L-lactate treatment; GC-1, mouse spermatogonial cell line; PI, propidium iodide; V-FITC, Annexin V-fluorescein isothiocyanate; LMD, laser measurement detector.

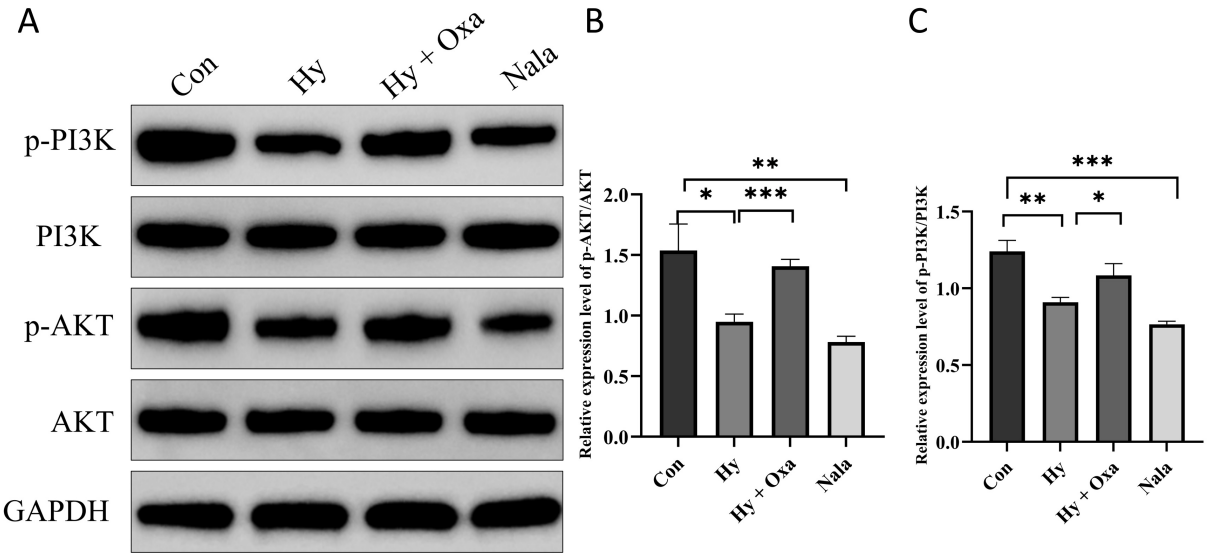


FIGURE 11. Western blot analysis of PI3K/AKT pathway activity in GC-1 cells under different treatments. Data are presented as mean ± SD (n = 3). (A) Representative immunoblots showing protein expression of phosphorylated PI3K (p-PI3K), total PI3K, phosphorylated AKT (p-AKT), total AKT, and GAPDH as a loading control. (B) Quantification of p-AKT/AKT. Compared with Con, Hy showed a significant decrease (*$p < 0.05$). Hy + Oxa partially reversed this effect, showing an increase compared with Hy (**$p < 0.01$). Nala markedly reduced p-AKT/AKT compared with Con (***$p < 0.001$). (C) Quantification of p-PI3K/PI3K. Compared with Con, Hy exhibited a significant reduction (**$p < 0.01$). Hy + Oxa significantly increased the ratio compared with Hy (*$p < 0.05$), though levels did not fully recover to those of Con. Nala further suppressed p-PI3K/PI3K relative to Con (***$p < 0.001$). Con, normoxia group; Hy, hypoxia group; Hy + Oxa, hypoxia + sodium oxamate; Nala, L-lactate treatment; p-PI3K, phosphorylated phosphatidylinositol 3-kinase; p-AKT, phosphorylated protein kinase B; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

oxamate can partially reverse this inhibition. Conversely, exogenous lactate under normoxic conditions mimics the effects of hypoxia, further supporting the conclusion that histone lactylation contributes to apoptosis in GC-1 cells, at least in part, by downregulating the PI3K/AKT survival pathway.

4. Discussion

This study examined the role of histone lactylation in hypoxia-induced apoptosis of GC-1cells and its underlying mechanisms. By testing different durations of hypoxia, we identified 36 h as the optimal point at which lactate accumulation and apoptosis were most evident. Hypoxia led to a marked increase in intracellular lactate, which enhanced histone lactylation and promoted apoptosis, as shown by increased Bax and Caspase-3 expression together with reduced Bcl-2. Both hypoxia and exogenous lactate suppressed PI3K/AKT signaling, whereas sodium oxamate alleviated this effect, indicating that histone lactylation contributes to apoptosis partly through downregulation of the PI3K/AKT pathway. These findings provide new insight into the epigenetic mechanisms linking hypoxia, lactate metabolism, and spermatogonial apoptosis.

4.1 Effects of hypoxia on male fertility and GC-1 cell apoptosis

The maintenance of male fertility depends on efficient spermatogenesis, a process highly vulnerable to hypoxic stress [32–35]. Hypoxia can impair testicular function and induce germ cell apoptosis [32, 33, 36], with spermatogonia being particularly susceptible [36]. To simulate this pathological condition, we established an *in vitro* hypoxia model using mouse GC-1 cells and observed a time-dependent induction of apoptosis. Our results showed that 36 h of hypoxia treatment reduced cell viability to 58% (Fig. 2), accompanied by upregulation of the pro-apoptotic genes *Bax* and Caspase-3 and downregulation of the anti-apoptotic gene Bcl-2 (Fig. 1). Prolonged hypoxia (48 h) exacerbated cellular damage (viability <50%), leading to extensive late apoptosis and decreased molecular stability, which compromised experimental reproducibility [37]. Therefore, we selected the 36-hour time point for subsequent mechanistic studies, as it allowed clear observation of early apoptotic events while maintaining cellular stability. Our cellular findings align with clinical observations of hypoxic spermatogenic failure, such as disrupted testicular microenvironment and germ cell loss [32, 33, 36] and provide a mechanistic model for understanding how hypoxia particularly triggers spermatogonial apoptosis, a key event leading to male infertility.

4.2 Role of lactate accumulation in hypoxia-induced spermatogonia apoptosis

Under hypoxic conditions, the metabolic shift toward anaerobic glycolysis leads to substantial lactate accumulation. While lactate serves as a vital energy substrate for male germ cells under physiological conditions [38–40], its role undergoes a

significant transition in pathological hypoxia. Our data showed that hypoxia significantly increased intracellular lactate levels in GC-1 cells by 1.9-fold (Fig. 3), indicating enhanced glycolytic activity. More importantly, lactate accumulation functioned not merely as a metabolic byproduct but as a pro-apoptotic signal: inhibition of lactate production using sodium oxamate , a lactate dehydrogenase inhibitor, effectively suppressed lactate generation and concurrently attenuated apoptosis.

This finding is consistent with recent studies indicating that lactate acts as a signaling molecule that induces cell death under various stress conditions [41–45]. Under physiological circumstances, lactate derived from Sertoli cells plays a crucial role in germ cell development [39]; however, our study reveals that under pathological hypoxia, lactate autonomously produced by spermatogonia switches to a pro-apoptotic factor. This mechanism is further supported by other research models. For instance, lactate accumulation during myocardial ischemia-reperfusion accelerates apoptosis [42], while in the nervous system, lactylation modifications have been shown to induce mitochondrial dysfunction and promote cell death [43, 44]. Therefore, we propose that lactate accumulation may act as a key metabolic switch mediating the transition from physiological function to pathological apoptosis in male germ cells under hypoxic conditions.

4.3 Lactate regulates GC-1 cell stress response through histone lactylation modifications

Having established that hypoxia induces lactate accumulation (Fig. 3), we next investigated whether this lactate serves as the precursor for histone lactylation (Kla) in GC-1 cells. Our data clearly demonstrate that hypoxic exposure robustly elevated both global histone lactylation and specific H3K18la modification, with H3K18la showing a particularly striking 12-fold increase compared to normoxic controls (Figs. 4,5). To unequivocally determine that lactate is the causative agent rather than a mere correlate, we performed functional perturbation experiments. Pharmacological inhibition of lactate production with sodium oxamate during hypoxia effectively suppressed the levels of both Pan-Kla and H3K18la. Conversely, and more importantly, supplementing exogenous sodium L-lactate under normoxic conditions was sufficient to recapitulate the hypoxic phenotype, markedly elevating histone lactylation to levels comparable to those seen in hypoxia (Figs. 5,6,7). This gain-of-function and loss-of-function evidence solidifies a direct causal relationship where lactate accumulation drives histone lactylation. This finding positions lactate as a critical metabolic-epigenetic transducer that translates hypoxic stress into a specific chromatin modification. This mechanism aligns with emerging roles of lactate in epigenetic regulation across various cell types, including microglia [30], cardiomyocytes [24, 46], neurons and glial cells [47], placental trophoblasts [24], and scleral fibroblasts [48], but our study is the first to definitively establish this lactate-Kla axis within spermatogonia. The nuclear localization of these lactylation signals (Figs. 6,7) further suggests their direct involvement in regulating transcriptional programs. This establishes the foundation for the subsequent pro-apoptotic effects.

4.4 Lactate promotes apoptosis in GC-1 cells through histone lactylation modifications

Our experiments demonstrated that expression of the apoptosis markers Bax and Caspase-3 was significantly elevated in the Hy group of GC-1 cells compared with the Con group, while Bcl-2 expression was notably reduced. This molecular profile was consistent with the increased apoptosis rate observed in the Hy group, indicating that hypoxia-induced lactate accumulation promotes apoptosis through activation of histone lactylation modifications. The reliability of these findings was further confirmed by functional assays: inhibition of histone lactylation with sodium oxamate effectively alleviated apoptosis in GC-1 cells, whereas supplementation with exogenous lactate under normoxic conditions reproduced the apoptotic phenotype observed in hypoxia. In addition, Western blot analysis revealed that the ratios of p-PI3K/PI3K and p-AKT/AKT were markedly decreased in the Hy group compared with the Con group. Inhibition of histone lactylation with sodium oxamate partially restored these ratios, while exogenous lactate under normoxia again reproduced the hypoxia-induced decreases. These findings suggest that lactate-mediated histone lactylation not only enhances the expression of pro-apoptotic genes but also suppresses the PI3K/AKT survival pathway, thereby further shifting the cellular balance toward apoptosis. Taken together, these results elucidate the mechanism by which lactate accumulation affects GC-1 cells in hypoxic environments and emphasize the importance of lactate as a regulator of histone lactylation in hypoxia-induced spermatogonial apoptosis. As a central mediator of metabolic stress, lactate may play a pivotal role in the hypoxic stress response of the male reproductive system. Future studies should therefore investigate the broader role of lactate and histone lactylation in both physiological and pathological processes, with particular attention to male infertility and testicular diseases, in order to assess their potential therapeutic value. In this regard, histone lactylation may represent a promising target for intervention and could provide a novel strategy for preventing hypoxia-induced germ cell death, offering new perspectives for the treatment of hypoxia-associated infertility and related disorders.

Bou-Gharios J *et al*. [37] demonstrated that lactate regulates histone acetylation and methylation in tumor cells. However, investigations into the role of histone lactylation in apoptosis under hypoxic conditions remain limited, particularly in the mouse spermatogonia cell line GC-1. The novelty of this study lies in its identification of lactate as a driver of spermatogonial apoptosis through histone lactylation under hypoxia, and in the validation of this modification's functional role using both lactate inhibition and exogenous lactate supplementation. Nonetheless, several limitations should be acknowledged. First, this work relied on a GC-1 cell model of hypoxia, which does not fully replicate the complexity of the *in vivo* testicular microenvironment; future studies incorporating animal models are required to confirm the role of histone lactylation in other cell types and tissues, with particular attention to its relevance for male reproductive physiology. Second,

whether histone lactylation can be targeted with specific inhibitors or regulators to treat hypoxia-induced reproductive dysfunction remains uncertain. The development of selective lactylation inhibitors may represent a novel therapeutic strategy for hypoxia-related diseases, providing a biological basis for new interventions. Finally, although our findings establish a strong correlation between elevated histone lactylation and apoptosis of GC-1 cells under hypoxia, direct mechanistic evidence is lacking, and the broader impact of lactylation on global transcription remains unexplored. Future studies employing high-throughput approaches such as RNA sequencing combined with chromatin immunoprecipitation (ChIP) will be essential to define the gene networks regulated by lactylation and to clarify the transcriptional programs through which this modification influences cell fate.

5. Conclusions

This study demonstrates that hypoxia-induced lactate accumulation promotes apoptosis in GC-1 cells through histone lactylation, with H3K18 identified as a key site of modification. Elevated intracellular lactate under hypoxic conditions enhances histone lactylation, which activates pro-apoptotic signaling while suppressing anti-apoptotic responses. At the same time, lactate accumulation modulates the PI3K/AKT signaling pathway, reducing the activity of survival proteins and thereby amplifying apoptotic progression. The reversal of these effects by inhibiting lactate dehydrogenase and their reproduction under normoxic lactate supplementation confirms the pivotal role of lactate in regulating cell fate through epigenetic mechanisms. Collectively, these findings provide new insight into the molecular mechanisms linking hypoxia, lactate metabolism, and spermatogonial apoptosis, and suggest that histone lactylation, through its regulation of both apoptotic pathways and the PI3K/AKT signaling axis, may represent a promising therapeutic target for hypoxia-induced male reproductive dysfunction.

AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

AUTHOR CONTRIBUTIONS

QT and XTL—designed the research study. XW—performed the research. TG—provided help and advice on the research. QT—analyzed the data. XW, QZ and LW—wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study involving GC-1 cells was reviewed and approved by the Ethics Committee of the 940th Hospital of the Joint Logistics Support Force of the Chinese People's Liberation Army (Approval No. 2023KYLL287). All experimental procedures were conducted in strict accordance with the approved research protocol and relevant ethical guidelines.

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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