# Histone lactylation: A new frontier in laryngeal cancer research (Review)

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**Abstract.** Laryngeal cancer is a common malignant tumor of the head and neck, and its occurrence and development are closely related to epigenetic modifications. Histone lactylation, a novel form of epigenetic modification, regulates gene expression and is involved in processes such as tumor metabolic reprogramming, shaping the tumor microenvironment, maintaining tumor stem cell characteristics and developing treatment resistance. The present review highlighted the molecular mechanisms and detection methods of histone lactylation and explores its potential role in laryngeal cancer, as it may serve as a valuable biomarker for diagnosis and prognosis. Furthermore, targeting the lactylation pathway may provide novel therapeutic strategies, with the potential to improve treatment outcomes for patients. Overall, understanding the role of histone lactylation in laryngeal cancer may potentially lead to innovative diagnostic and therapeutic approaches and improve the management of laryngeal cancer in the future.

**Contents**



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## 1. Introduction

Laryngeal cancer is one of the common malignant tumors in the head and neck, which accounts for 20-30% of all types of head and neck tumors (1). Squamous cell carcinoma is the most common pathological type of laryngeal cancer (2). With the impact of factors such as environmental pollution, smoking, alcohol consumption and changes in lifestyle, the incidence and mortality of laryngeal cancer have been rising (3). According to the latest statistics, the incidence and mortality rates of global laryngeal cancer are 3.9 per 100,000 and 2.1 per 100,000, respectively (4). Although comprehensive treatment methods including surgery, radiotherapy and chemotherapy continue to improve, the prognosis of patients with laryngeal cancer remains unsatisfactory, particularly for advanced patients who exhibit a low 5-year survival rate (5). Thus, there is an urgent need to further explore the molecular mechanisms underlying the occurrence and development of laryngeal cancer to identify new diagnostic markers and therapeutic targets.

The importance of epigenetics in tumor research is increasingly evident, and histone modifications are considered one of the core mechanisms of epigenetic regulation (6). Among various histone post-translational modifications (PTMs), traditional modifications such as acetylation, methylation, phosphorylation and ubiquitination have been systematically studied (7-10). However, the emerging histone lactylation modification has recently drawn attention, especially for its potential importance in tumor metabolism and immune regulation (11). In contrast to acetylation, lactylation not only derives from the metabolic product lactate but also directly reflects changes in the cellular metabolic state, which possesses a unique dynamic and reversible nature (12).

Previous studies on lactylation modification in various squamous cell carcinomas (such as oral squamous cell carcinoma and esophageal squamous cell carcinoma (ESCC) indicated that by regulating gene expression, lactylation modification participates in tumor metabolic reprogramming, immune evasion and therapeutic resistance (Table I) (13-19). According to the study reported by Wang *et al* (18), histone H3K9 lactylation serves a key role in immune evasion in head and neck squamous cell carcinoma (HNSCC). These studies

provide an important reference for investigations on the role of lactylation modification in laryngeal cancer. The present review highlighted the molecular basis of histone lactylation modification and associated research in laryngeal cancer, which explored its potential applications in the diagnosis, prognostic evaluation and treatment of laryngeal cancer.

## 2. Epigenetic regulatory mechanisms of laryngeal cancer

Epigenetics serve a key role in the initiation and progression of laryngeal cancer and other malignant tumors in the ear, nose and throat (ENT) field. Previous studies indicated that the development of laryngeal cancer and other types of HNSCC is often accompanied by notable epigenetic abnormalities. These changes are not direct mutations of the DNA base sequence but manifest at the levels of DNA methylation, histone modification and non-coding RNA regulation, which affect cell fate by modulating gene expression (3,20,21).

As an example of DNA methylation, tumor suppressor genes such as p16INK4a, MGMT and DAPK1 frequently exhibit promoter hypermethylation in tumor tissues of patients with laryngeal cancer, which leads to transcriptional silencing (22-24). This 'epigenetic inactivation' can inhibit apoptosis and promote aberrant proliferation, which represents a key molecular mechanism in laryngeal carcinogenesis and disease progression. Furthermore, alterations in DNA methylation status are closely associated with tumor prognosis and therapeutic response that serve as important references for molecular subtyping and prognostic assessment (25). PTMs are similarly a key mechanism of epigenetic regulation in ENT tumors (26). Dysregulated histone deacetylases and methyltransferases readily result in oncogene activation and tumor suppressor gene silencing, which thereby drive tumor development. A previous study has shown that the expression levels of H4R3me2a in laryngeal cancer cells are closely associated with their proliferative, migratory and invasive capacities (27). Non-coding RNAs, especially microRNAs (miR) such as miR-21 and miR-155 and long non-coding RNAs such as HOX transcript antisense intergenic RNA, are aberrantly expressed in laryngeal cancer tissues and can further promote tumor progression and metastasis by regulating key tumor suppressors or downstream pathway genes, which affects patient prognosis (28-30). In addition, lifestyle factors such as smoking and alcohol consumption (31,32) and metabolic status, as well as exogenous factors including Epstein-Barr virus and human papillomavirus infection (33,34) may subsequently influence host epigenetics and the risk of associated malignancies, through alterations in the microbiome (35).

At the functional level, epigenetic modifications regulate gene expression by affecting chromatin structure. For example, histone acetylation generally leads to chromatin relaxation and promotes transcriptional activation; conversely, deacetylation compacts chromatin and represses gene expression (36). Histone methylation exerts distinct functions depending on the modification site; H3K4me3 is predominantly associated with gene activation (37), whereas H3K27me3 commonly mediates gene silencing (38). Other modifications, such as phosphorylation and ubiquitination, also participate in various biological processes, including the cell cycle, DNA damage repair and chromosome remodeling (39,40).

In summary, epigenetic modifications serve a key role in the initiation, progression and treatment tolerance of ENT malignancies, particularly laryngeal cancer. Multiple modifications, including PTMs and DNA methylation, together constitute a refined gene expression network. A thorough elucidation of the aberrant epigenetic mechanisms in laryngeal cancer not only contributes to the refinement of molecular tumor subtyping and the development of novel diagnostic and targeted therapeutic approaches, but also potentially provides a theoretical foundation and clinical prospects for the exploration of innovative therapies and the enhancement of precision medicine.

## 3. Molecular mechanisms of histone lactylation modification

*Source and metabolism of lactate*. Lactate is the end product of glycolysis, primarily generated under hypoxic conditions in normal cells (41). However, in tumor cells, even under oxygen-sufficient conditions, the glycolytic pathway remains highly active, a phenomenon known as the 'Warburg effect' (42). This metabolic reprogramming enables tumor cells to rapidly uptake glucose and convert it into lactate and thus meets the energy and metabolic intermediate requirements for rapid proliferation (43).

Lactic acid not only functions intracellularly but is also actively transported out of the cell, which serves as a signaling molecule involved in the regulation of various biological processes, particularly epigenetic regulation (44,45). For example, in breast cancer, lactate promoted angiogenesis by regulating the expression of hypoxia-inducible factor-1-α (46). In colorectal cancer, lactate suppressed antitumor immune responses by regulating the expression of immunosuppressive factors such as transforming growth factor-β (TGF-β) (47).

*Discovery and characteristics of histone lactylation modification*. Histone lactylation was first reported by Zhang *et al* (12) in 2019 as a novel form of epigenetic modification. The histone lactylation modification is formed through the covalent binding of lactate molecules to histone lysine residues and is one of the acylation modifications of lysine. Previous studies have reported that the histone lactylation modification mainly occurs at specific sites on histone H3, such as H3K18 (48,49). The discovery of histone lactylation expanded the current understanding of epigenetic modifications, as it suggests that lactate is not only a metabolic byproduct but can also directly participate in the regulation of gene expression.

The specificity and functions of lactylation modification may vary with cell type and environmental conditions. For example: i) in breast cancer cells, expression levels of H3K18la were markedly elevated, which promoted the expression of key transcription factors for tumor cell growth (50); ii) in ESCC, elevated H3K9la expression levels enhanced tumor cell invasion and metastasis (16); and iii) in healthy cells, lactylation modification levels were relatively low and mainly participate in the maintenance of metabolic homeostasis (51). These studies indicated that lactylation modification is dynamic and reversible, capable of quickly responding to changes in cellular metabolic status.

Table I. Lactylation in types of squamous cell carcinoma.

A, Oral squamous cell carcinoma

| First author, year | Proteins modified by lactylation | Affected genes or pathways | Role of lactic acidification | (Refs.) |
|---|---|---|---|---|
| Jing *et al*, 2024 | DHX9 K146 | Not specified. | Promoted the occurrence and progression of oral squamous cell carcinoma. | (13) |
| Huang *et al*, 2024 | Histone lysine | BCAM | Promoted invasion, angiogenesis and chemoresistance in oral squamous cell carcinoma. | (14) |
| Song *et al*, 2024 | SF3A1, hnRNPA1, hnRNPU and SLU7 | Spliceosome, ribosome and glycolysis/gluconeogenesis pathway | Lactylation levels were negatively associated with the prognosis of patients with oral squamous cell carcinoma. | (15) |

B, Esophageal squamous cell carcinoma

| First author, year | Proteins modified by lactylation | Affected genes or pathways | Role of lactic acidification | (Refs.) |
|---|---|---|---|---|
| Zang *et al*, 2024 | H3K9la | LAMC2 | Promoted LAMC2 expression under hypoxic conditions, enhanced cell proliferation. | (16) |
| Fu *et al*, 2024 | H3K18la | c-Myc | Promoted the proliferation of squamous cell carcinoma. | (17) |

C, Head and neck squamous cell carcinoma

| First author, year | Proteins modified by lactylation | Affected genes or pathways | Role of lactic acidification | (Refs.) |
|---|---|---|---|---|
| Wang *et al*, 2024 | H3K9la | IL-11; JAK2/STAT3 | Promoted $CD8^+$ T cell dysfunction and poor immunotherapy response. | (18) |

D, Cervical squamous cell carcinoma

| First author, year | Proteins modified by lactylation | Affected genes or pathways | Role of lactic acidification | (Refs.) |
|---|---|---|---|---|
| Huang *et al*, 2024 | H3K18la | GPD2 | Mediated M2 macrophage polarization and promoted the malignant progression of cervical cancer. | (19) |

DHX9, DExH-box helicase 9. BCAM, basal cell adhesion molecule; SF3A1, splicing factor 3A subunit 1; hnRNPA1, heterogeneous nuclear ribonucleoprotein A1; hnRNPU, heterogeneous nuclear ribonucleoprotein U; SLU7, splicing factor, lethal (2) underexported in drosophila 7; LAMC2, laminin subunit γ-2; H3K, histone H3 lysine; JAK, janus kinase; GPD2, glycerol-3-phosphate dehydrogenase 2.

*Regulatory mechanisms of histone lactylation modification*. Regulation of histone lactylation involves multiple molecular mechanisms, but its detailed enzymatic regulatory network remains to be elucidated. Compared with classical PTMs such as acetylation, methylation and phosphorylation, lactylation exhibits a lower basal expression level and stronger tissue specificity, which indicates that under normal physiological conditions it is largely restricted to specific cell types and their metabolic activity (52-55) (Table II). As a bridge between metabolic signaling and epigenetic regulation, histone lactylation can dynamically respond to changes in intracellular lactate concentration and couple metabolic state with the regulation of gene expression (56).

In terms of enzyme-mediated modification, the formation of lactylation may be catalyzed by lactate dehydrogenase (LDH) or other enzymes, such as acyl-CoA synthetase short-chain family member 2 and acyl-CoA synthetase family member 2 (44,57-59). LDH is a key enzyme in the glycolytic pathway, whose primary function is to catalyze the reduction of pyruvate to lactate (60). Previous studies have demonstrated

Table II. Comparison of histone lactylation modifications with other modification types.

| Characteristic | Type of modification | | | |
|---|---|---|---|---|
| | Lactylation | Acetylation | Methylation | Phosphorylation |
| Modification substrate | Lactate | Acetyl-CoA | SAM | ATP |
| Target amino acid | Lysine | Lysine | Lysine, arginine | Serine, threonine, tyrosine |
| Regulatory enzyme system | Not fully elucidated, mechanisms incomplete | HAT/HDAC | HMT/HDM | Kinase/phosphatase |
| Metabolic sensitivity | Very high, closely related to glycolysis | High, affected by energy metabolism | Moderate dependence on metabolism | Low to moderate |
| Distribution characteristics | Tissue-specific, predominantly in metabolically active cells | Ubiquitous | Ubiquitous | Ubiquitous |
| Degree of variation | Low, changes with metabolic adaptation | Moderate to high, flexible changes | Moderate, both activation and repression | High, rapid response |
| Physiological function | Reflect cellular metabolic state, links glycolysis with gene expression, regulates inflammation and cellular adaptation | Relaxes chromatin structure, promotes gene transcription | Activates/represses gene expression, regulates gene silencing and imprinting | Involved in chromosome condensation, cell cycle regulation, stress response and DNA damage repair |

SAM, S-adenosylmethionine; HAT, histone acetyltransferase; HDAC, histone deacetylase; HMT, histone methyltransferase; HDM, histone demethylase.

that LDH, by regulation of the intracellular lactate concentration, directly or indirectly affects the occurrence of lactylation modification (12). A recent study has also reported that alanyl-tRNA synthetase 1 and alanyl-tRNA synthetases 2 have been identified as intracellular L-lactate sensors and lactate transferases, which catalyzes the ATP-dependent binding of L-lactate to lysine and forms lactylation modifications (61).

Regarding the delactylation process, specific delactylases have not yet been clearly identified. However, some studies suggested that the delactylation process may involve certain members of the deacylase family. The sirtuin (SIRT) family, which consists of nicotinamide adenine dinucleotide-dependent deacetylases, is known to serve key roles in the regulation of histone acetylation and other modifications (62). For example, SIRT3 has been found to possess delactylation activity, with its activity against histone H4K16la being notably higher compared with that of other SIRT family members (including SIRT1, SIRT2, SIRT4, SIRT5, SIRT6 and SIRT7) (63). Additionally, SIRT2 has been reported to have 'eraser' functions in neuroblastoma (64). Future research is warranted to further explore the specific types of delactylases and their functions in tumors.

Lactylation modification also interacts with other epigenetic modifications (such as acetylation, methylation and phosphorylation) to jointly regulate gene expression (65). For example, lactylation modification may compete with acetylation for the same lysine sites, which affects gene transcription activity (64). Furthermore, lactylation might influence chromatin openness or compaction through its interaction with methylation modifications. This complex network of modifications provides a molecular basis for the multiple roles of lactylation in tumors and its potential functions in tumors characterized by pronounced metabolic abnormalities, such as laryngeal cancer, warrant in-depth investigation in future research.

## 4. Potential roles of histone lactylation modification in laryngeal cancer

*Tumor metabolic reprogramming.* Similar to other tumor cells, laryngeal cancer cells exhibit markedly enhanced glycolysis; even in the presence of sufficient oxygen, tumor cells tend to rely on glycolysis for energy production, a manifestation of the Warburg effect (66). This metabolic reprogramming leads to massive lactate accumulation, which then acts as a signaling molecule to regulate gene expression through histone lactylation modification. Previous studies have demonstrated that lactylation modification can activate the expression of metabolism-related genes [such as glucose transporter type 1 (GLUT1) and lactate dehydrogenase A (LDHA)], which further enhances the activity of the glycolytic pathway (67,68). This positive feedback mechanism allows tumor cells to quickly acquire energy and metabolic intermediates to meet their rapid proliferative demands.

In addition, lactylation modification may enhance tumor metabolic reprogramming by regulating the expression of key genes involved in other metabolic pathways, such as lipid metabolism and amino acid metabolism (69,70). For example, lactylation may activate the expression of fatty acid synthase and glutamine transporter 5, thereby enhances tumor cell lipid

synthesis and amino acid metabolism capacity (71,72). These metabolic adaptations not only provide the material basis for tumor cell proliferation but also enhance their tolerance to adverse environments.

*Shaping the tumor microenvironment*. Lactate functions not only within tumor cells but also influences the shaping of the tumor microenvironment when secreted extracellularly. A previous study has reported that in HNSCC, lactate regulates the expression of the IL-11 gene through H3K9la and subsequently activates the Janus kinase 2/STAT3 signaling pathway, which leads to $CD8^+$ T cell exhaustion, and ultimately enables tumor cells to evade immune surveillance (18). This finding indicated that lactate can inhibit antitumor immune responses by altering the function of immune cells in the tumor microenvironment. Furthermore, lactylation modification may also promote tumor-associated inflammatory responses by regulating the expression of inflammatory cytokines (73). These cytokines can not only attract immunosuppressive cells (such as myeloid-derived suppressor cells and regulatory T-cells) into the tumor microenvironment but also supports tumor cell proliferation and metastasis by promoting angiogenesis and matrix remodeling (74).

Cancer-associated fibroblasts (CAFs) are an essential component of the tumor microenvironment; the activated state CAFs is closely associated to tumor invasiveness and metastatic capability. Lactylation modification can regulate the secretion of cytokines and growth factors [such as TGF-β and vascular endothelial growth factor (VEGF)] by CAFs, which thereby promote tumor cell invasion and angiogenesis (75). Additionally, lactylation modification may lead to acidification of the tumor microenvironment, which further enhances the invasiveness and therapeutic resistance of tumor cells (76).

*Cancer stem cell characteristics*. Cancer stem cells, a small population of cells within tumors that possess self-renewal capabilities and multipotent differentiation potential, are considered the primary driving force for tumor invasion, metastasis and recurrence (77). Lactylation modification may enhance the stem cell properties of laryngeal cancer cells by regulating the expression of stemness-related genes [such as the stem cell transcription factor Sox2 and Octamer-binding transcription factor 4 (OCT4)] (78,79). Sox2 and OCT4 are key transcription factors in maintaining the stem cell state; high expression levels of Sox2 and OCT4 are closely associated with tumor cell invasiveness and metastatic potential and lactylation modification may activate the expression of these genes, which endows laryngeal cancer cells with stronger migratory and invasive capacities compared to adjacent normal tissue cells or precancerous lesion cells (80,81). Furthermore, lactylation modification might further enhance cancer stem cell characteristics by regulating the key genes of signaling pathways such as Wnt/β-catenin, Notch and Hedgehog (82). These pathways serve important roles in the maintenance and function of cancer stem cells and their abnormal activation leads to increased tumor invasiveness and therapeutic resistance (83). By modulating the expression of these key genes, lactylation modification may endow laryngeal cancer cells with enhanced adaptability and survival capacity, which promotes malignant progression.

*Chemotherapy and radiotherapy resistance*. Lactylation modifications may enhance the tolerance of laryngeal cancer cells to chemotherapy and radiotherapy through multiple mechanisms. Firstly, lactylation modifications can regulate the expression of DNA damage repair genes, such as radiation-sensitive genes [such as RAD51 recombinase (RAD51)] (84). RAD51 is a key protein in the homologous recombination repair pathway; high expression of RAD51 effectively repairs DNA double-strand breaks induced by chemotherapeutic drugs or radiotherapy and thereby protects tumor cells from the lethality of DNA damage (85). A previous study has demonstrated that inhibiting GLUT-1 with siRNA can reduce the expression of RAD51 and DNA-dependent protein kinase catalytic subunit, which weakens the DNA repair capacity and thereby increases apoptosis to enhance the radiosensitivity of laryngeal cancer stem cells (86). Secondly, lactylation modification may enhance tumor cell tolerance to oxidative stress induced by radiotherapy by regulating the expression of antioxidant genes, which thus helps cancer cells evade treatment-induced oxidative damage (44,87). In addition, lactylation modification might further enhance chemoresistance by modulating the expression of cell cycle-related genes, which delays cell cycle progression and reduces the killing effect of chemotherapeutic drugs on rapidly dividing cells (88,89).

## 5. Clinical significance of histone lactylation modification

*Diagnostic and prognostic biomarkers*. Research has indicated that the level of lactylation modification is associated with tumor malignancy, invasiveness and patient survival (90). The enhanced glycolysis (Warburg effect) in laryngeal cancer cells leads to lactate accumulation, which markedly increases the level of histone lactylation modification. Therefore, detecting the levels of lactylation modification in patient tissues or blood samples potentially holds promise as an important basis for the early diagnosis and assessment of laryngeal cancer in the future. In the diagnostic context, specific sites of histone lactylation modification can serve as candidate molecular markers for laryngeal cancer. Therapeutically, the dynamic changes in lactylation modification may reflect the metabolic state and epigenetic characteristics of the tumor, which provides a reference for personalized treatment. For prognostic evaluation, high levels of histone lactylation may associate with more aggressive and metastatic tumors and lower patient survival rates, whereas lower levels may indicate improved prognosis (91,92). Furthermore, the detection of lactylation modifications can be combined with other biomarkers such as programmed cell death-ligand 1 (PD-L1) to establish a multi-biomarker diagnostic and prognostic assessment system, which thereby enhances the accuracy of cancer diagnosis and the predictive capability of prognosis (93,94).

*Therapeutic targets*. Given the important roles of lactylation modification in tumor metabolic reprogramming, shaping the tumor microenvironment, maintaining cancer stem cell characteristics and therapeutic resistance, targeting lactate metabolism pathways or enzymes associated with lactylation modification may potentially offer novel strategies for laryngeal cancer treatment in the future.

First, drugs targeting lactate metabolism (such as LDHA inhibitors) hold notable therapeutic potential. LDHA is a key enzyme in the glycolytic pathway that catalyzes the conversion of pyruvate to lactate, which directly influences lactate accumulation and the occurrence of lactylation modification (68). LDHA inhibitors, by reducing lactate production and lowering levels of histone lactylation, can suppress tumor metabolic reprogramming and immune evasion. For example, the LDHA inhibitor FX11 has markedly inhibited the growth of various tumors, including pancreatic cancer, breast cancer and melanoma (95-97), and its application in laryngeal cancer warrants further investigation.

Second, targeting enzymes associated with lactylation modification (both the lactylation 'writers' and potential 'erasers') may provide novel intervention strategies for laryngeal cancer. Lactylation enzymes (writers) are responsible for adding lactyl groups to target proteins, while de-lactylating enzymes (erasers) can remove the lactyl groups that have already been added to the proteins (98). Although the specific enzymes catalyzing lactylation and those responsible for delactylation have not been fully elucidated, ongoing studies have suggested that the dynamic regulation of lactylation serves a key role in tumor development and progression (98). By developing inhibitors against the enzymes that catalyze lactylation, it may be possible to block the occurrence of lactylation modification, which thereby inhibits tumor cell proliferation and invasion. If specific delactylases can be identified in the future, inducing their activity could potentially reverse lactylation effects and suppress malignant tumor progression.

Finally, lactylation modification may also serve as a target for combination therapies, integrating with existing immunotherapy, chemotherapy and radiotherapy regimes (99). For example, lactylation modifications promote tumor immune evasion by regulating the expression of PD-L1 (100). Thus, targeting lactylation modification could enhance the efficacy of immune checkpoint inhibitors (such as anti-PD-1/PD-L1 antibodies) (101). Furthermore, combining LDHA inhibitors with chemotherapeutic drugs could simultaneously inhibit tumor metabolism and DNA damage repair, which thereby increases chemosensitivity (102). As research on lactylation modification deepens, its potential in the clinical application for laryngeal cancer will gradually be revealed, which offers novel prospects for precision diagnosis and treatment for patients with laryngeal cancer.

## 6. Detection methods for histone lactylation modification

*Mass spectrometry analysis*. Mass spectrometry is one of the core techniques for studying histone lactylation modification and is primarily used to identify specific lactylation sites and quantify their modification levels (103). Owing to its high sensitivity and resolution, mass spectrometry can precisely detect the lactylation modification on histone lysine residues. Researchers typically utilize liquid chromatography-tandem mass spectrometry, in combination with proteolytic digestion and peptide separation methods, to conduct in-depth analyses of histone samples (12). Furthermore, mass spectrometry allows for quantitative analysis of dynamic changes in lactylation under different conditions. By comparing the levels of histone lactylation modifications in normal vs. tumor cells, the potential role of lactylation modification in tumorigenesis and progression may be elucidated (92). Additionally, mass spectrometry can be combined with other omics techniques (such as metabolomics and transcriptomics) to explore the association between lactylation modification, cellular metabolism and gene expression (104). Despite its advantages, mass spectrometry faces certain technical challenges; for example, the chemical properties of lactylation are similar to those of acetylation, which may lead to ambiguity in identifying modification sites (65). Furthermore, lactylation modifications are typically of low abundance, which necessitates the development of higher-resolution mass spectrometers and optimized sample preparation protocols in the future (105).

*Specific antibody detection*. Specific antibody detection is another important method for the study of histone lactylation modification, primarily used to assess the levels and distribution of lactylation modifications (106,107). In recent years, specific antibodies targeting particular lactylation sites (such as H3K18la and H3K23la) have been developed, which provide key tools for the detection of lactylation modifications (65). These antibodies can recognize the unique chemical structure of lactylation, which enables highly specific detection. In practice, researchers often use western blot techniques for quantitative analysis of changes in lactylation modifications under different experimental conditions (108); immunofluorescence techniques allow visualization of the spatial distribution of lactylation modifications within the nucleus (91). Additionally, co-immunoprecipitation techniques can be employed in conjunction with specific antibodies to study the interactions between lactylation modifications and other proteins or epigenetic modifications (109). However, despite the advantages of ease of operation and high sensitivity, specific antibody detection also has limitations. For example, non-specific binding of antibodies may affect data reliability (110). Furthermore, the limited availability of commercial antibodies for lactylation modifications restricts their application in large-scale studies.

*Gene editing technologies*. Gene editing technologies, especially the CRISPR/Cas9 system, provide powerful tools for exploring the functions of histone lactylation modification (111). With CRISPR/Cas9, researchers can knock out or overexpress genes associated to lactylation modification (such as LDHA and LDHB) to investigate the specific role of lactylation in cellular metabolism, gene expression and disease (112). The applications of CRISPR/Cas9 in this context include several aspects. First, by knocking out enzymes associated to lactylation modification, one can study the mechanisms of lactylation formation and its impact on cellular functions (113). Second, CRISPR/Cas9-mediated site-directed mutagenesis can be used to investigate the regulatory role of specific histone lactylation sites (such as H3K18 and H3K23) on gene expression and cell behavior (114). Furthermore, CRISPR/Cas9 can be combined with other omics technologies to systematically explore the functions of lactylation modification in various biological processes (115). Although CRISPR/Cas9 technology holds broad application prospects, challenges remain in its use for lactylation

research. The dynamic and complex nature of histone lactylation might complicate the interpretation of gene editing results. Additionally, the off-target effects of CRISPR/Cas9 could impact the accuracy of experimental outcomes, which necessitates the optimization of experimental designs and further validation of results (116).

Mass spectrometry analysis, specific antibody detection and gene editing technologies constitute the three core methods for the study of histone lactylation modification. The integrated application of these methods not only provides important support for the basic research of lactylation modification but also lays the foundation for its clinical translation in disease diagnosis and treatment. With ongoing technological advancements, these methods will further advance in-depth research into lactylation modification.

## 7. Conclusion and outlook

Histone lactylation modification, as a novel epigenetic regulatory mechanism, serves key roles in tumor metabolic reprogramming, shaping the tumor microenvironment, maintaining cancer stem cell characteristics and mediating therapeutic resistance. Although research on the role of histone lactylation modification in laryngeal cancer is still in its early stages, the aforementioned studies have indicated that lactylation modification regulates gene expression and is involved in the occurrence and development of laryngeal cancer. This provides novel research directions and potential targets for the diagnosis, prognostic evaluation and treatment of laryngeal cancer. Future research may focus on elucidating the molecular regulatory mechanisms of lactylation modification, its interactions with other epigenetic modifications and its specific functions in laryngeal cancer. Furthermore, the development of targeted detection tools and therapeutic agents against lactylation modification will offer technical support and pave the way for clinical translation in the precise diagnosis and treatment of laryngeal cancer. Combining basic research with clinical applications, lactylation modification could become novel avenue of research in the study of laryngeal cancer, which may potentially provide novel treatments for patients in the future.

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## Authors' contributions

QiaT and CH wrote the manuscript. QiaT created the tables. QizT and ZZ revised the manuscript. CH supervised the research. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

## Ethics approval and consent to participate

Not applicable.

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## Competing interests

The authors declare that they have no competing interests.

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