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Research article

A pan-cancer multi-omics analysis of lactylation genes associated with tumor microenvironment and cancer development

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ABSTRACT

Background: Lactylation is a significant post-translational modification bridging the gap between cancer epigenetics and metabolic reprogramming. However, the association between lactylation and prognosis, tumor microenvironment (TME), and response to drug therapy in various cancers remains unclear.

Methods: First, the expression, prognostic value, and genetic and epigenetic alterations of lactylation genes were systematically explored in a pan-cancer manner. Lactylation scores were derived for each tumor using the single-sample gene set enrichment analysis (ssGSEA) algorithm. The correlation of lactylation scores with clinical features, prognosis, and TME was assessed by integrating multiple computational methods. In addition, GSE135222 data was used to assess the efficacy of lactylation scores in predicting immunotherapy outcomes. The expression of lactylation genes in breast cancers and gliomas were verified by RNA-sequencing.

Results: Lactylation genes were significantly upregulated in most cancer types. CREBBP and EP300 exhibited high mutation rates in pan-cancer analysis. The prognostic impact of the lactylation score varied by tumor type, and lactylation score was a protective factor for KIRC, ACC, READ, LGG, and UVM, and a risk factor for CHOL, DLBC, LAML, and OV. In addition, a high lactylation score was associated with cold TME. The infiltration levels of $CD8^+$ T, γδT, natural killer T cell (NKT), and NK cells were lower in tumors with higher lactylation scores. Finally, immunotherapy efficacy was worse in patients with high lactylation scores than other types.

Conclusion: Lactylation genes are involved in malignancy formation. Lactylation score serves as a promising biomarker for predicting patient prognosis and immunotherapy efficacy.

1. Introduction

Cancer is a substantial worldwide public health issue that profoundly impacts human well-being and quality of life [1–7]. Despite

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its escalating societal impact, the efficacy of cancer treatment outcomes remains inadequate, necessitating further investigation into its pathogenesis and potential therapeutic targets. Lactic acid, previously considered a waste product of anaerobic metabolism, has been found to possess various novel biological functions such as serving as a carbon source, signaling molecule, and immunomodulator [8,9]. In the context of cancer and fast-growing cells, lactate plays a crucial role in the glycolysis-dependent reprogramming of energy metabolism [10]. Furthermore, lactate has the ability to facilitate crucial oncogenic mechanisms such as angiogenesis, invasion, metastasis, and immune evasion through its influence on the acidic tumor microenvironment (TME) [11]. Recently identified lactate-induced modifications of lactylation are an important component of lactate function [12]. Lactylation modifications can alter the spatial conformation of chromatin, affect DNA accessibility, and regulate gene transcription to promote tumorigenesis [13]. The modification of histone lactylation has been observed to facilitate the progression of clear cell renal cell carcinoma by activating the transcription of PDGFRβ [14]. Moreover, SIRT2-mediated histone delactylation was found to suppress the proliferation and migration in neuroblastoma [15,16]. However, studies on lactylation are still in their infancy, and the association between lactylation and tumorigenesis has not been systematically analyzed, indicating the need for further investigation.

Immunotherapy signifies a significant advancement in the realm of cancer therapy, as it leverages the patient's immune system to combat malignant cells and exhibits enduring clinical efficacy against a wide range of cancers [17–19]. However, the efficacy of immunotherapy is largely dependent on the TME of the patient, which includes stromal cells (fibroblasts and immune cells), cancer cells, and extracellular components and is regulated by various factors such as cytokines, growth factors, and chemical mediators [20,21]. The induction of lactylation by lactic acid plays a crucial role in the preservation of tissue homeostasis [11]. This process effectively governs the infiltration of cancer-associated fibroblasts (CAF), tumor-infiltrating myeloid cells (TIM), and tumor stem cells (CSCs) within the microenvironment, thereby facilitating the remodeling of the TME and fostering tumor advancement [22]. A recent study found that lactylation-driven methyltransferase-like 3 (METTL3)-mediated RNAm^{6}A modification promotes the sustained immunosuppressive activity of TIM [23]. Tregs, as pivotal cells in mediating immunosuppression, are also implicated. The lactylation of MOESIN induced by lactic acid regulates the production of Treg cells by enhancing TGF-β signaling, thereby promoting tumorigenesis [24]. Therefore, a comprehensive analysis of the association between lactylation genes and TME may offer a novel reference point for tumor immunotherapy and prognosis.

Lactylation has been proposed as a new therapeutic target for oncology [25]. Histone lactylation promotes ocular melanoma development by activating the m^{6}A reader protein YTHDF2, suggesting that histone lactylation is a potential target for the treatment of ocular melanoma. However, a systematic study examining the impact of Lactylation on clinical outcomes across all types of cancer has yet to be conducted. The development of high-throughput sequencing technology has facilitated the extraction of features from extensive cancer data, enabling pan-cancer analysis [26–34]. The objective of this study was to investigate the expression profile, prognostic significance, genomic mutations, and drug sensitivity of lactylation genes across 33 distinct tumor types through a pan-cancer analysis. Additionally, the association between the lactylation score and patient prognosis, TME and response to immunotherapy was examined. This study provides a comprehensive overview of lactylation genes at the pan-cancer level.

2. Methods and materials

2.1. Data acquisition

The lactylation gene dataset was obtained from the study of Liu, Xuelian et al. [10]. This review comprehensively summarizes recent advances in the discovery, derivation, cross-species landscape, and diverse functions of lactylation. Expression profile data and clinical characteristics information of The Cancer Genome Atlas (TCGA) and the Genotype-Tissue Expression (GTEx) pan-cancer samples were retrieved from UCSC XENA (https://xenabrowser.net/datapages/). Lactylation gene phase single nucleotide variation (SNV) analysis and gene copy number variation (CNV) data were obtained from the Gene Set Cancer Analysis (GSCA) database (http://bioinfo.life.hust.edu.cn/GSCA/#/).

2.2. Pan-cancer differential expression and protein interaction analysis of lactylation genes

Differential gene expression analysis between tumor and normal tissues was performed utilizing the "limma" R package, with *p*-values and logFC values presented by heat maps. The correlation analysis between lactylation genes was conducted utilizing the "corplot" R package. GeneMANIA is a website designed to build protein-protein interaction (PPI) networks, providing gene function prediction hypotheses and identifying comparable genes [35]. This research constructed a PPI network of proteins encoding lactylation genes using GeneMANIA to explore the interactions between proteins encoding lactylation genes.

2.3. Construction of a lactylation-associated prognostic model for kidney renal clear cell carcinoma (KIRC)

In order to examine the prognostic implications of lactylation genes in KIRC, a lactylation-related model was constructed employing the least absolute shrinkage sum selection operator (LASSO) regression technique, which was implemented using the "glmnet" R package. The risk score for each KIRC patient was determined by applying the subsequent formula: Risk score = ΣCoefi × Genei, where Coefi represents the coefficient and Genei denotes the expression level of the corresponding lactylation gene. Based on the median risk score, KIRC patients were categorized into two subgroups: the low-risk subgroup and the high-risk subgroup. The overall survival (OS) was compared using Kaplan-Meier (K-M) analysis, while the accuracy of the prediction model was tested using the receiver operating characteristic (ROC) curve.

2.4. Pan-cancer survival analysis of the lactylation genes

Univariate Cox regression analysis and log-rank test were performed for the lactylation gene in pan-cancer, and *p*-value and HR value were extracted. HR value > 1 was considered a risk factor, HR < 1 was considered a protective factor, and a heat map presented the results.

2.5. Lactylation scores

Lactylation scores for each sample of each tumor in TCGA were quantified by the ssGSEA method of the "GSVA" R package. The "ggpubr" R package was utilized to show the differences in scores in paired tumors and paracancerous tissues and variance in lactylation scores of patients with different stages in different tumors. The relationship between lactylation scores and OS of patients, disease-specific survival (DSS), disease-free interval (DFI), and progression-free interval (PFI) in different tumors was analyzed by univariate Cox regression with the "forestplot" R package. In addition, the "survival" R package was used to identify the best cutoff value based on the smallest p-value. Tumor samples were then divided into high and low groups according to this cutoff value. K-M curves demonstrated the differences in prognosis between high and low-scoring groups in different tumors.

2.6. Gene set variation analysis (GSVA)

GSVA is a parameter-free and unsupervised method for estimating the variation in pathway activity within a population sample [36]. The GSVA gene set is derived from the dataset of the MsigDB database, which encompasses 50 hallmark pathways. The association between the lactylation score and the hallmark pathway score was computed for each tumor, and the outcomes were visualized using the "pheatmap" R package.

2.7. Analysis of tumor immune microenvironment

Immune score, stromal score, and estimate score of tumor tissues were quantified utilizing the "ESTIMATE" R package, and the results were presented by heat map and radar plot. Immune cell infiltration data were acquired from the TIMER2.0 database (http://timer.cistrome.org/) to analyze the correlation between lactylation scores and immune cell infiltration. Additionally, the ImmuCellAI database (http://bioinfo.life.hust.edu.cn/ImmuCellAI#!/) was employed to evaluate the association between lactylation scores and immune cell abundance in distinct tumor types. Subsequently, a co-expression analysis was conducted to explore the correlation between lactylation scores and immunomodulatory-related genes, encompassing immune activation genes, major histocompatibility complex (MHC) genes, chemokines, and chemokine receptors.

2.8. Correlation analysis of lactylation score and response to immunotherapy

Data for the immunotherapy cohort GSE135222 were retrieved from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/). K-M survival curves demonstrated the differences in the prognosis between immunotherapy patients with high lactylation scores and those with low lactylation scores. In addition, the relationship between the lactylation score and the efficacy of immunotherapy was assessed by comparing the proportion of patients with disease progression after treatment. The ROC curve was developed by the "pROC" R package to predict the effect of immunotherapy by lactylation score and immune checkpoint PDCD1, CTLA4, and CD274 gene expression. The area under the ROC curve (AUC) was also measured.

2.9. Drug sensitivity analysis

Drug sensitivity data were acquired from the Genomics of Drug Sensitivity 2 (GDSC2) database (https://www.cancerrxgene.org/) and the Cancer Therapeutics Response Portal (CTRP) database (https://portals.broadinstitute.org/ctrp/) to obtain drug sensitivity data for human cell lines. Additionally, Spearman correlation analysis was carried out to obtain correlations between lactylation gene mRNA expression and drug IC50 values.

2.10. Validation of the lactylation genes in glioma

The 26 cases of breast cancer tissues and paracancerous tissues were obtained from the First Affiliated Hospital of Wenzhou Medical University (Zhejiang, China). The HMO6 and U251 were cultured in DMEM. All medium were supplemented with 10% fetal bovine serum (FBS) and 1% Penicillin/Streptomycin. Total RNA was isolated by TRIzol reagent (TaKaRa, Dalian, China). RNA integrity was detected by an Agilent 2100 bioanalyzer and the RNAs were sequenced on an Illumina platform. The immunohistochemical images were procured from the Human Protein Atlas (HPA) database.

2.11. Statistical analysis

The results of RNA-sequencing were analyzed using a *t*-test and all other statistical analyses were processed by R software (version 4.1.1). $P < 0.05$ were deemed as a statistically significant value.

3. Results

3.1. Dysregulation of lactylation genes and their relation with prognosis in multiple cancer types

As per the TCGA and GTEx datasets, the expression levels of lactylation genes in 31 kinds of TCGA tumors and normal tissues were compared, and the dysregulation patterns of different cancer types and genes were identified. The resulting heat map revealed a significant upregulation of lactylation genes in most cancers, with GLO1, HDAC2, HDAC8, and HDAC1 exhibiting the most pronounced dysregulation (Fig. 1A). In addition, all lactylation genes were significantly overexpressed in thymoma (THYM), pancreatic adenocarcinoma (PAAD), and brain lower grade glioma (LGG), while most lactylation genes were not significantly altered in pheochromocytoma and paraganglioma (PCPG) and sarcoma (SARC). A robust positive correlation was observed in the expression of almost all lactylation genes across various types of cancer, as indicated by the correlation coefficients. This discovery suggests a widespread co-expression relationship among lactylation genes (Fig. 1B). Therefore, the interaction pattern of lactylation genes was further investigated. The protein interaction network map revealed that lactylation genes encode proteins with complex interactions with each other (Supplementary Fig. 1A).

Given the significant involvement of lactylation genes in cancer, an investigation was conducted to examine the association between lactylation genes and patient prognosis. In the context of pan-cancer, a univariate Cox regression analysis revealed that a majority of lactylation genes exhibited prognostic relevance across multiple cancer types (Fig. 1C). This correlation varied by cancer type, and high expression of specific lactylation genes in different cancer types may be associated with a good prognosis or lead to poor outcomes. For example, HDAC2 is a risk factor for poor prognosis in up to six malignancies, including mesothelioma (MESO), SARC, adrenocortical carcinoma (ACC), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), and breast invasive carcinoma (BRCA). Moreover, increased HDAC2 expression is linked with good prognosis in individuals with KIRC and rectum adenocarcinoma

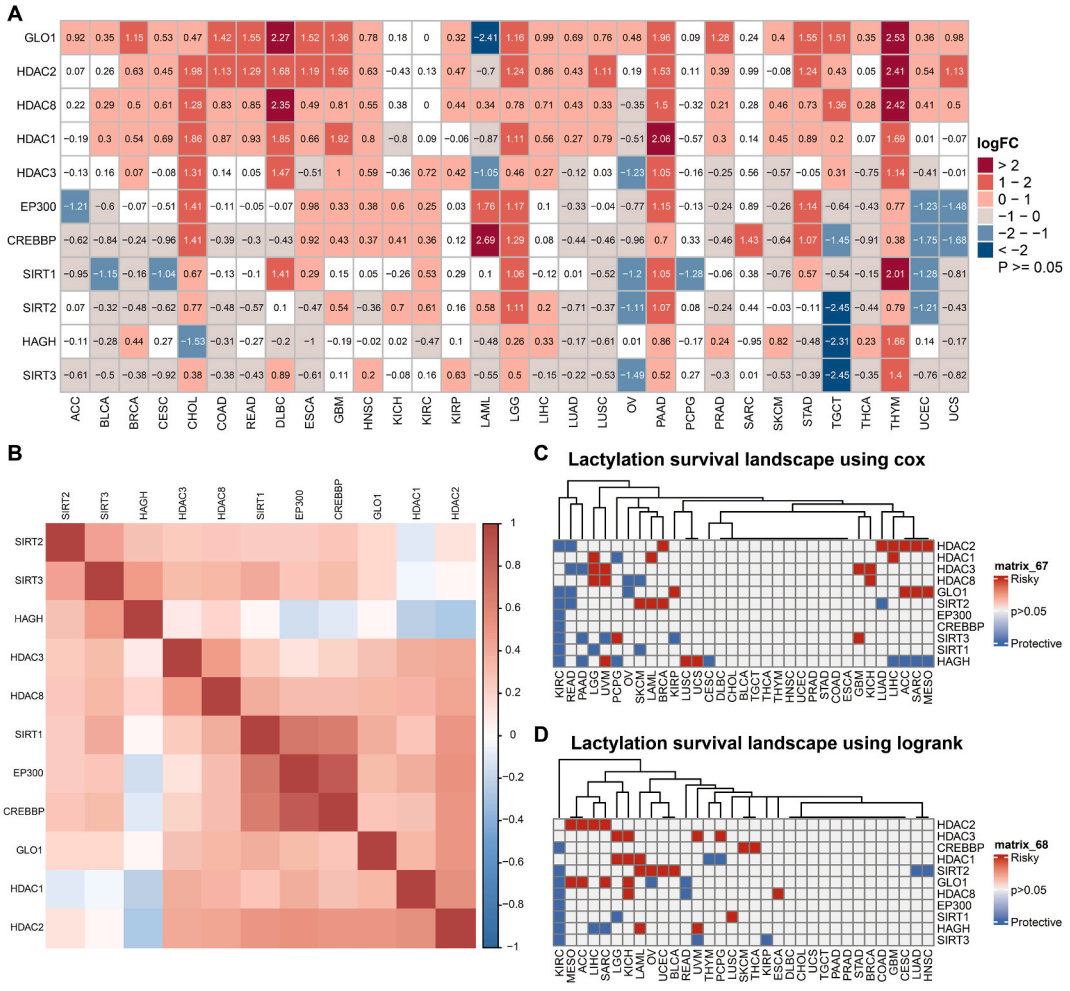

Fig. 1. Transcriptome landscape and prognostic analysis of lactylation genes. (A) Heat map showing differential mRNA expression of lactylation genes between 31 tumor types and adjacent normal tissues. (B) Correlation matrix of lactylation gene expression in pan-cancer. Overall survival analysis of lactylation genes in 33 tumors from TCGA via (C) univariate cox regression analysis and (D) logrank analysis.

(READ). Log-rank analysis was carried out to further investigate the correlation between lactylation genes and pan-cancer survival. The findings highlighted that lactylation genes were significantly linked with OS in multiple cancers (Fig. 1D). Among them, elevated expression of HDAC2 and HDAC3 was an important risk factor for four different forms of cancer, while high expression of SIRT3 was linked to a good prognosis in three cancers. The findings of this study indicate a strong association between lactylation genes and survival in patients with KIRC, as demonstrated by both Cox regression and log-rank analysis. Furthermore, the majority of lactylation genes were found to have a protective effect on KIRC, highlighting the significance of these genes in the context of this particular cancer. In conclusion, these results reveal the pattern of lactylation gene dysregulation in various cancer types. Moreover, the effect of lactylation genes on patient prognosis varies by cancer type.

Next, we constructed a risk model for KIRC based on lactylation genes (Supplementary Figs. 2A–B, Supplementary Table 1). The KIRC patients were then categorized into high-risk and low-risk groups based on the median risk score (Supplementary Fig. 2C). It was observed that patients in the low-risk group exhibited a higher survival rate and longer survival time compared to those in the high-risk group (Supplementary Fig. 2D). Furthermore, the ROC curve analysis demonstrated that the risk score had a high predictive accuracy, as evidenced by a 5-year area under the ROC curve (AUC) of 0.725 (Supplementary Fig. 2E). Hence, the lactylation-related features identified in this study hold promise as novel indicators for KIRC prognosis.

3.2. Somatic mutational landscape of the lactylation gene

SNV data from 10,234 patients with 33 cancer types was then analyzed to detect the frequency of deleterious mutations in each cancer subtype. Tumor types with a high frequency of SNV were uterine corpus endometrial carcinoma (UCEC), skin cutaneous melanoma (SKCM), bladder urothelial carcinoma (BLCA), cervical squamous cell carcinoma and endocervical adenocarcinoma

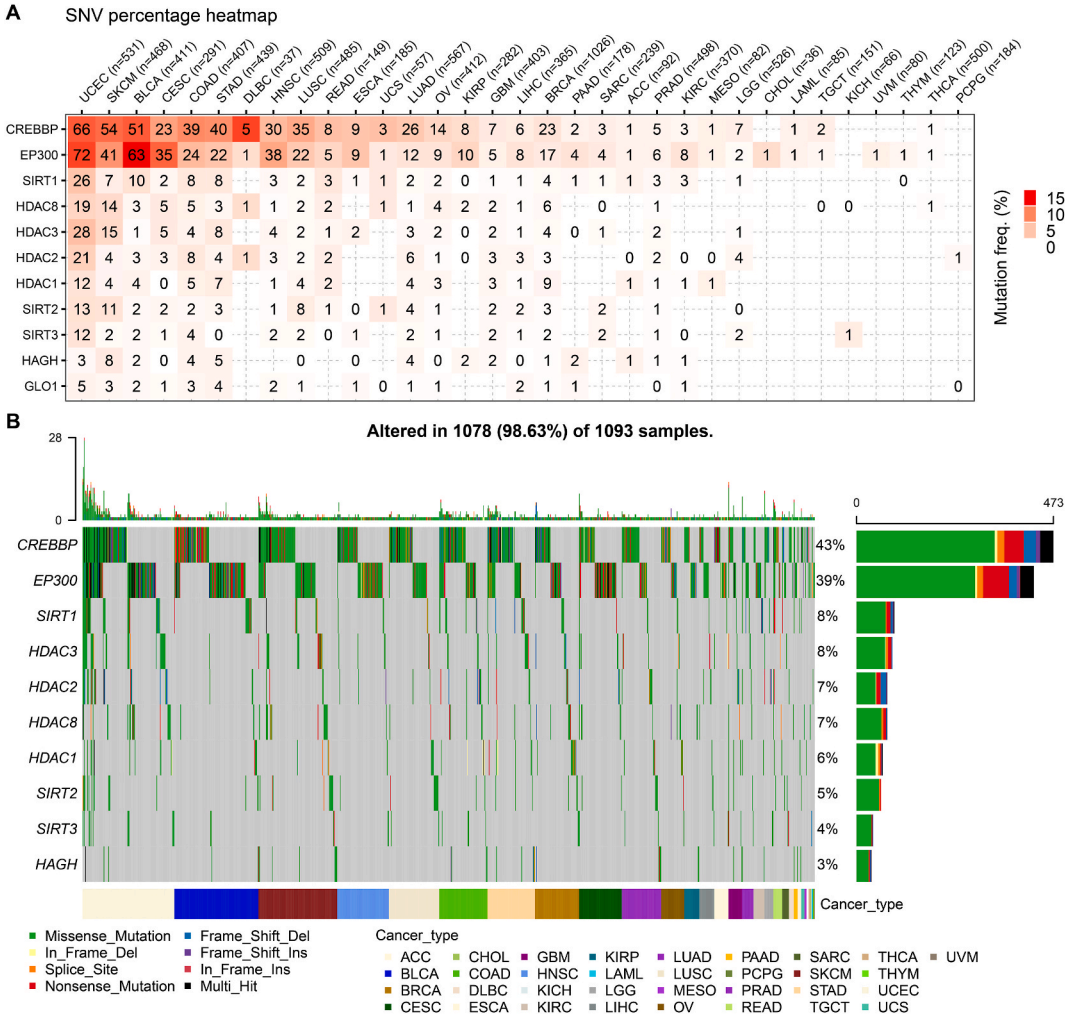

Fig. 2. Single nucleotide variation (SNV) and somatic mutation analysis of lactylation genes. (A) SNV profiles of lactylation genes in each tumor type. (B) Oncoplot depicting the distribution of mutations in the top 10 mutated genes in the selected cancer sample set.

(CESC), colon adenocarcinoma (COAD), and stomach adenocarcinoma (STAD), while cholangiocarcinoma (CHOL), acute myeloid leukemia (LAML), testicular germ cell tumors (TGCT), kidney chromophobe (KICH), uveal melanoma (UVM), THYM, thyroid carcinoma (THCA), and PCPG had almost no detectable SNV (Fig. 2A). For lactylation genes, CREBBP and EP300 had the highest mutation frequencies, while HAGH and GLO1 had lower mutation frequencies in the cancer types. In addition, the mutation distribution waterfall plot showed that the top 10 mutated genes were CREBBP (43%), EP300 (39%), SIRT1 (8%), HDAC3 (8%), HDAC2 (7%), HDAC8 (7%), HDAC1 (6%), SIRT2 (5%), SIRT3 (4%), and HAGH (3%). As per the variant classification, missense mutations were the most common SNVs (Fig. 2B). In addition, single nucleotide polymorphisms (SNPs) were the predominant type of mutations, with the highest frequencies occurring in C > T and C > A transitions, followed by T > C reversals (Supplementary Figs. 3A–F).

3.3. CNV and methylation analysis of lactylation genes

Somatic CNVs are widely present in human cancers and are thought to drive tumorigenesis. The CNV pie chart distribution revealed the presence of CNVs for the lactylation gene across all 33 TCGA cancer types analyzed. Notably, these CNVs were observed at higher frequencies in ACC, uterine carcinosarcoma (UCS), BRCA, SARC, LUAD, STAD, CHOL, SKCM, esophageal carcinoma (ESCA), lung squamous cell carcinoma (LUSC), TGCT, BLCA, ovarian serous cystadenocarcinoma (OV), and KICH. Conversely, they occurred less frequently in LAML, THCA, THYM, and prostate adenocarcinoma (PRAD) (Fig. 3A). Per CNV stratification, gene heterozygous amplification and heterozygous deletion were the most prevalent CNVs, while pure amplification and deletion occurred less frequently. In addition, correlation analysis revealed a significant and positive association between lactylation gene mRNA expression and CNV in the majority of cancers (Fig. 3B). In addition, the methylation differences of the lactylation gene between tumor and normal samples were explored. The methylation of lactylation genes was heterogeneous in different tumors (Fig. 3C). For example, CREBBP showed high methylation levels in kidney renal papillary cell carcinoma (KIRP), UCEC, and BRCA while displaying low methylation levels in head and neck squamous cell carcinoma (HNSC) and KIRC. Correlation analysis highlighted that lactylation gene mRNA expression was negatively associated with methylation levels in most cancers.

3.4. Panorama of lactylation scores

The ssGSEA method, implemented through the R package “GSVA," was utilized to quantify the lactylation scores of each cancer type within the TCGA cohort. The results showed small differences in lactylation scores between tumor types, with LAML having the highest lactylation scores and mesothelioma (MESO) having the lowest scores (Fig. 4A). Subsequently, a comparison was made between the lactylation scores of paired tumor tissues and their corresponding paracancerous tissues. The expression of the lactylation score was considerably lower in BRCA, ESCA, HNSC, KIRP, LIHC, THCA, and STAD than in normal tissues, while in PRAD, the lactylation score was higher than in normal tissues (Supplementary Fig. 4). Furthermore, the correlation between the lactylation score and tumor stage was explored. The outcomes indicated that the lactylation score was negatively associated with the stage of certain

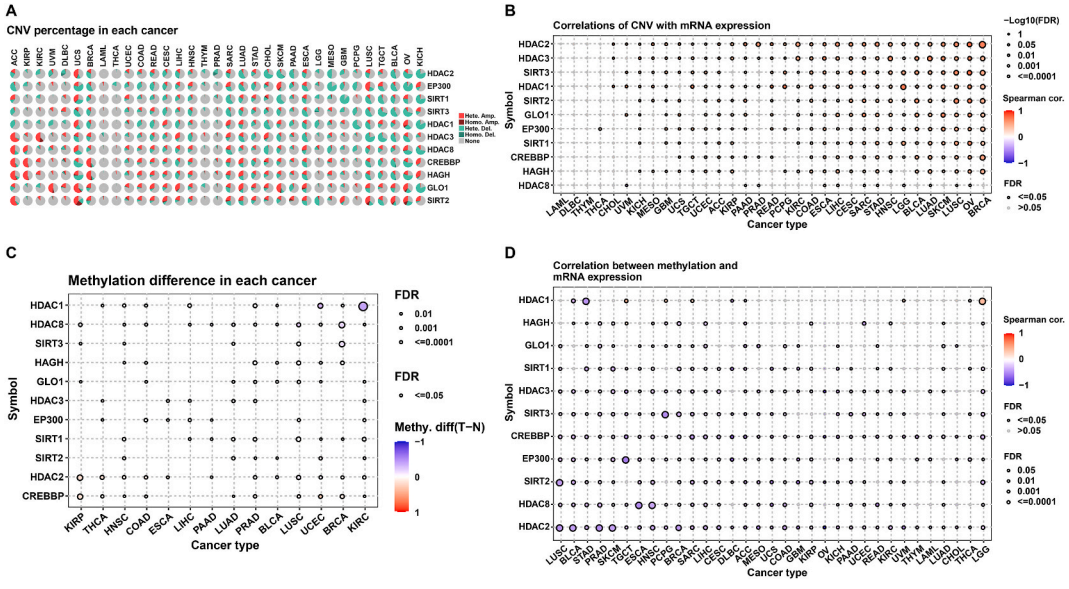

Fig. 3. Copy number variation (CNV) analysis and methylation levels of lactylation genes. (A) Heterozygous and homozygous CNV profiles of lactylation genes in each tumor type, including the percentage of deletion and amplification. Hete Del: heterozygous deletion; Hete Amp: heterozygous amplification; Homo Del: homozygous deletion; Homo Amp: homozygous amplification. (B) Correlations of CNV with lactylation gene mRNA expression. (C) Methylation differences of lactylation genes between tumor and normal samples. (D) Correlations between methylation and lactylation gene mRNA expression.

cancers, such as ACC, BLCA, COAD, KICH, KIRC, LUAD, SKCM, and THCA (Supplementary Figs. 5A–I). However, it is noteworthy that lactylation scores were higher in individuals with stage II tumors than in individuals with stage I tumors in UCS.

3.5. Survival analysis of lactylation score

A univariate Cox regression analysis examined the correlation between lactylation score and patient prognosis. The outcomes highlighted that the lactylation score was associated with a good prognosis for various cancers. Specifically, for OS, the lactylation score was a protective factor for KIRC, ACC, READ, LGG, UVM, and LUAD (Fig. 5A). For DSS, the lactylation score was a protective factor for KIRC, ACC, UVM, LGG, READ, THYM, and BRCA (Fig. 5B). For DFI, the lactylation score was a protective factor for BRCA (Fig. 5C). For PFI, the lactylation score was a protective factor for KIRC, LGG, PAAD, glioblastoma multiforme (GBM), and ACC and a risk factor for PCPG (Fig. 5D). A K-M analysis was also performed to further confirm the predictive value of the lactylation score. Individuals in the TCGA training cohort were assigned to either the high- or low-risk group based on the best cutoff value. The results showed that high lactylation scores were linked with favorable survival in ACC, GBM, HNSC, KICH, KIRC, LGG, LIHC, LUAD, MESO, PAAD, PCPG, READ, STAD, THYM, UCS, and UVM (Fig. 6A-T). In contrast, in CHOL, lymphoid neoplasm diffuse large B-cell lymphoma (DLBC), LAML, and OV, patients with high lactylation scores had poor prognoses. These results suggest two patterns of lactylation score-related survival in different cancer types.

3.6. GSVA of lactylation scores

To investigate potential pathways related to lactylation, the association between lactylation score and signature pathway score was calculated in each tumor (Fig. 7A). Significant positive correlations were found between lactylation scores and protein secretion, mitotic spindle, PI3K/Akt/mTOR signaling, Hedgehog signaling, Wnt/β-catenin signaling, Myc targets V1 and heme metabolism. Additionally, significant negative correlations were found with inflammatory response, allograft rejection, and IL-6/JAK/STAT3 signaling, coagulation, interferon gamma response, IL2/STAT5 signaling, complement, interferon alpha response, xenobiotic metabolism, and TNFa signaling via NF-κB pathways. Notably, higher lactylation scores were associated with lower scores in “IL-6/JAK/STAT3 signaling", “interferon gamma response", and “IL2/STAT5 signaling", suggesting that lactylation scores were closely related to immune regulation-related pathways.

3.7. Correlation of lactylation scores with cold TME

Accumulating evidence indicates that lactylation is involved in the regulation of tumor immunity [8]. In addition, prior research has shown a strong correlation between GSVA and lactylation scores, as well as immunoregulation-related pathways. Therefore, the relationship between lactylation scores and TME was investigated in more depth. Initially, the correlation between the lactylation score and the estimated proportion of immune and stromal cells in the TME was examined in 33 tumor samples from TCGA. Fig. 8A–E demonstrated that the lactylation score exhibited a notable negative association with the stromal score, estimate score, and immune score across most cancers. Additionally, it displayed a substantial positive correlation with tumor purity, except in the cases of LAML, PAAD, STAD, and READ.

Next, the link between lactylation scores and the infiltration of different immune cell types in each cancer type was analyzed. Based on different algorithms of the TIMER2 database, it was repeatedly found that lactylation scores were strongly negatively correlated

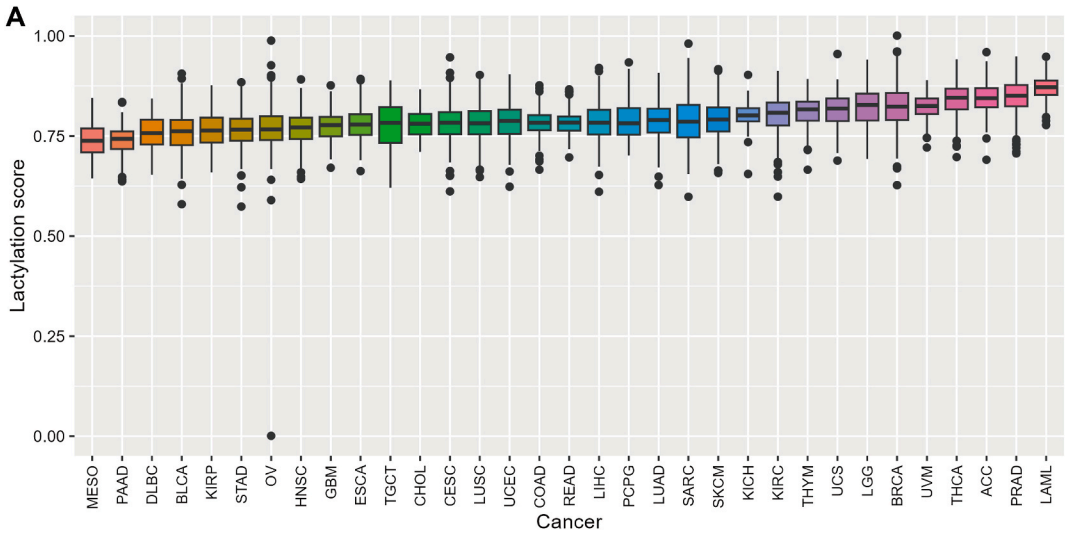

Fig. 4. (A) Distribution of lactylation scores for different cancer types based on the TCGA dataset.

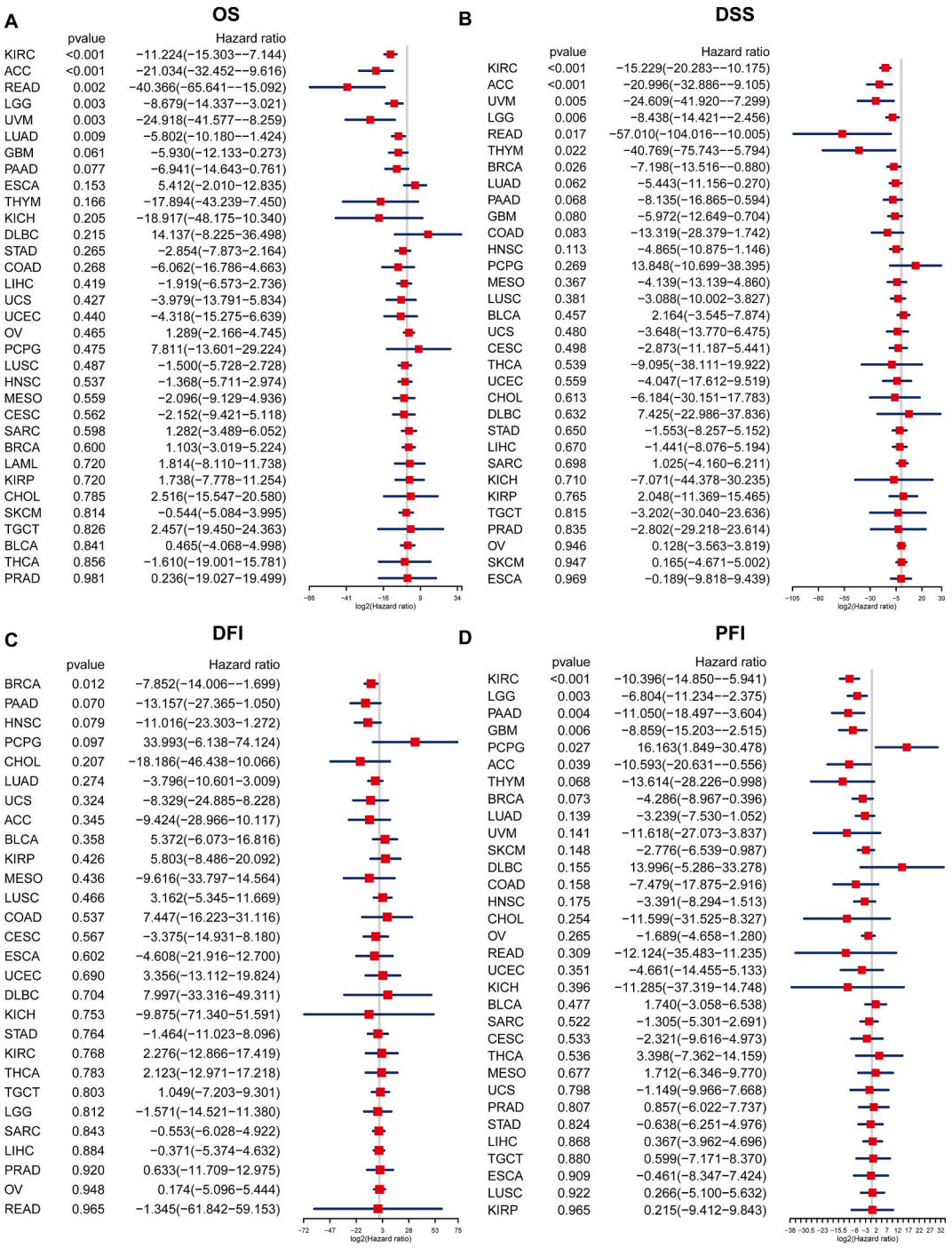

Fig. 5. Relationship between lactylation scores and patient prognosis in different tumors. Correlations between lactylation scores and (A) overall survival (OS), (B) disease-specific survival (DFS), (C) disease-free interval (DFI), and (D) progression-free interval (PFI) in various tumors determined by univariate cox regression analysis.

with macrophages, $CD8^+$ T, γδT, natural killer T (NKT), dendritic cells, and cancer-associated fibroblasts at the pan-cancer level, especially notable in ACC, UCS, UCEC, THYM, TGCT, SARC, MESO, LUAD, BRCA, BLCA (Fig. 9A). Therefore, it can be inferred that there is a strong correlation between high lactylation scores and a cold TME. Analysis of the ImmuCellAI database revealed a significant negative correlation between lactylation scores and tumor immune cell infiltration scores (Fig. 10A). In addition, almost all cancer types showed that the lactylation score was negatively associated with Tex, NK, Tc, $CD8^+$ T, and NKT cell infiltration.

Additionally, the correlation between lactylation score and immune activation genes (Fig. 11A), MHC genes (Fig. 11B), chemokine

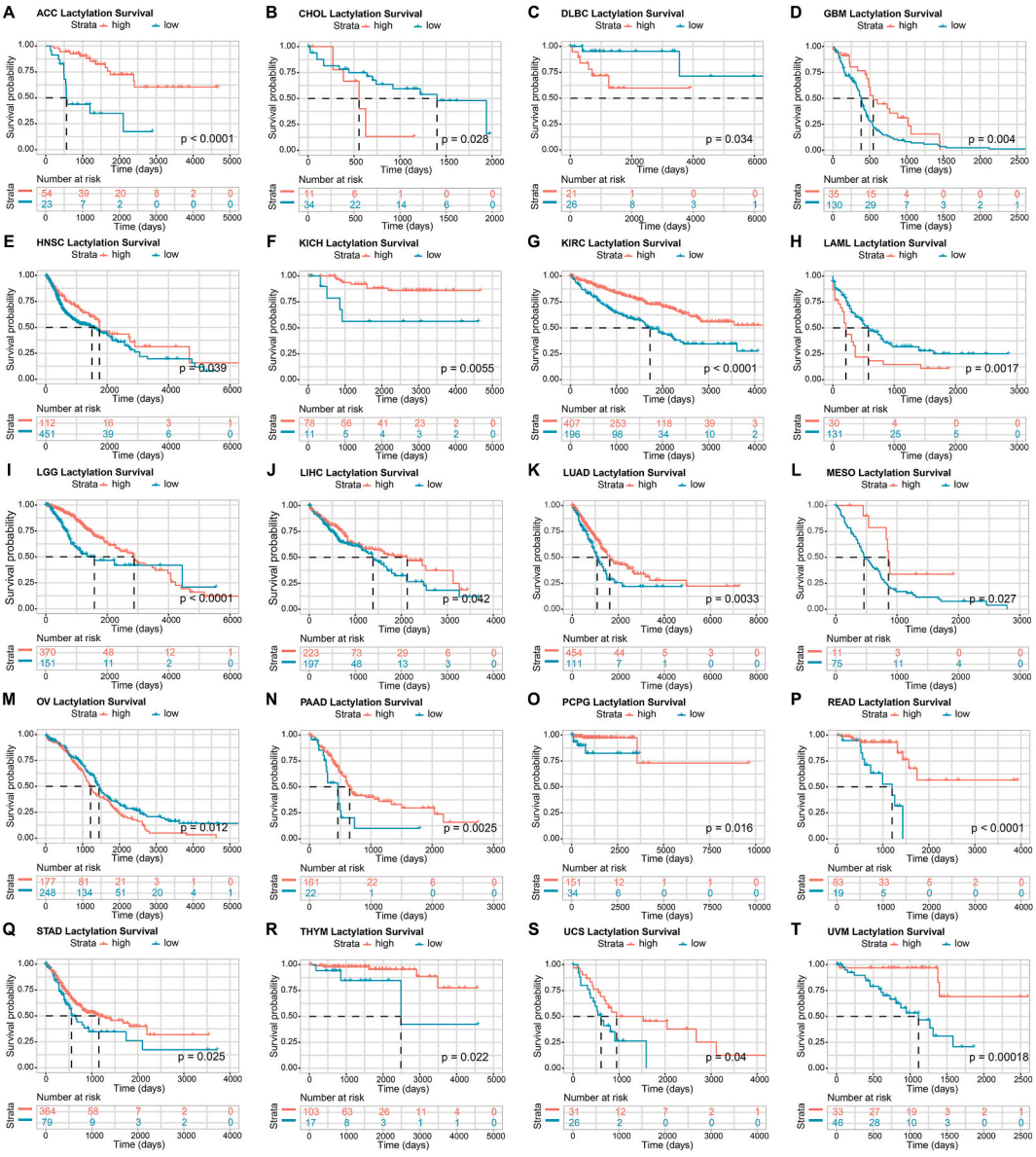

Fig. 6. Kaplan-Meier curves were used to analyze the relationship between lactylation scores and patient prognosis in (A) ACC, (B) CHOL, (C) DLBC, (D) GBM, (E) HNSC, (F) KICH, (G) KIRC, (H) LAML, (I) LGG, (J) LIHC, (K) LUAD, (L) MESO, (M) OV, (N) PAAD, (O) PCPG, (P) READ, (Q) STAD, (R) THYM, (S) UCS, (T) UVM.

receptors (Fig. 11C), and chemokines (Fig. 11D) was evaluated. Except for OC, DLBC, THYM, KIRC, LAML, READ, HNSC, STAD, and LUAD, lactylation scores were negatively correlated with most immunoregulatory genes in other tumors. In conclusion, high lactylation scores were associated with cold TME, showing negative regulation of immune-related pathways, lower levels of immune cell infiltration, and lower expression of immunoregulatory factors.

3.8. Association of lactylation score with patient response to immunotherapy

During the investigation of the attributes of the TME that are correlated with the lactylation score, a notable correlation was observed between a high lactylation score and a cold TME. This discovery prompted an examination of the potential impact of the lactylation score on the efficacy of immunotherapy. Patients were stratified according to lactylation score based on data from the GSE135222 non-small cell lung cancer (NSCLC) cohort. K-M survival analysis highlighted that a high lactylation score was considerably linked to worse OS ($p = 0.022$, Fig. 12A). In addition, a higher proportion of individuals in the high lactylation score group experienced disease progression after treatment with immune checkpoint blockade (ICB) therapy (Fig. 12B). Lactylation scores were

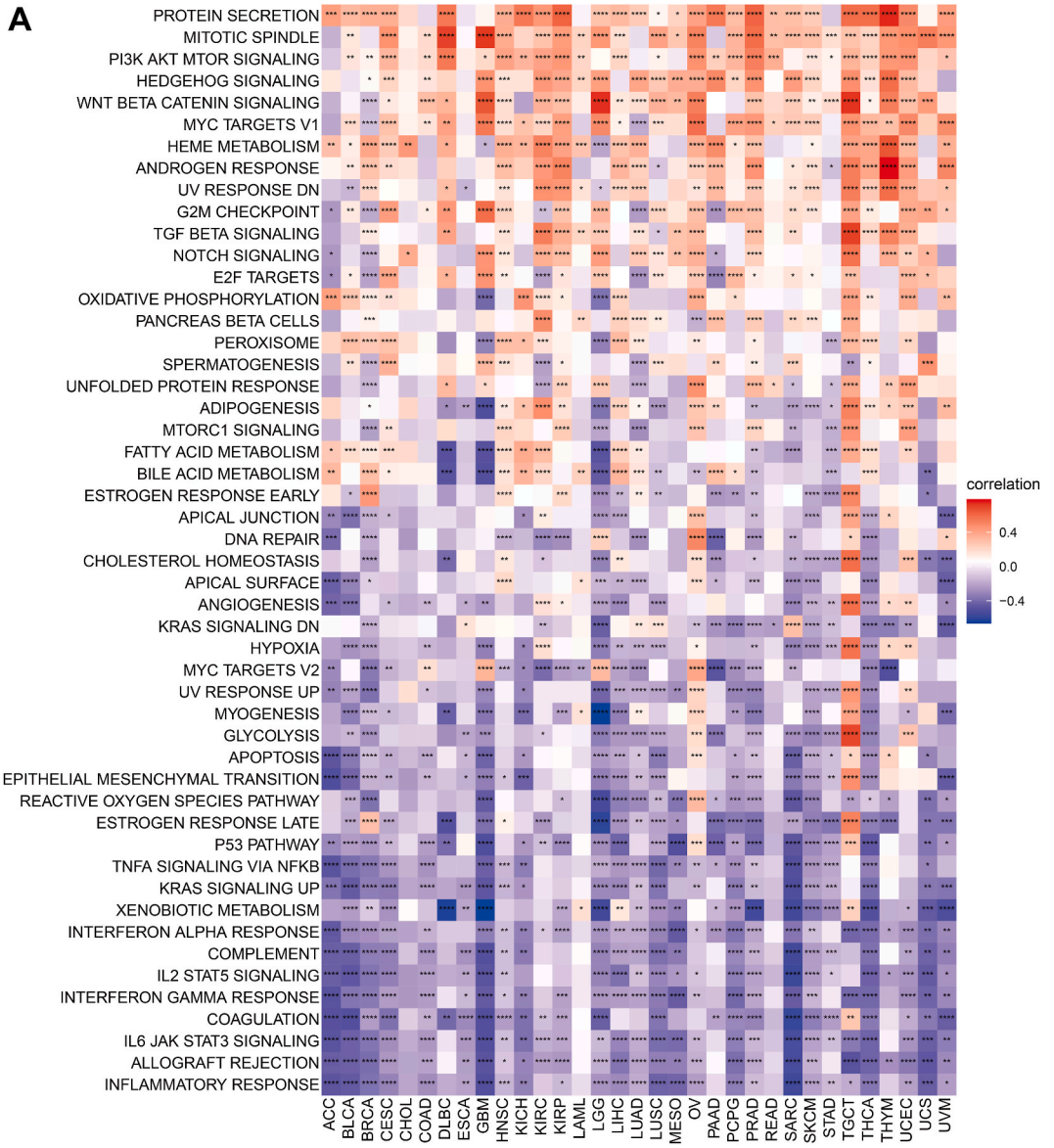

Fig. 7. (A) Heat map showing the correlation between lactylation scores and 50 hallmark pathways in the MsigDB database.

also considerably higher in patients in the disease progression group than in the non-progression group (Fig. 12C). Finally, a comparison of the predictive effect of different biomarkers was conducted using ROC curves. The AUC value of the lactylation score was 0.778, which had a better predictive effect than other immune checkpoints (PDCD1, CTLA4, and CD274) (Fig. 12D). In conclusion, it was found that individuals with high lactylation scores had worse immunotherapy efficacy, and the lactylation score could be an excellent biomarker to predict immunotherapy efficacy.

3.9. Correlation of lactylation genes with drug sensitivity

Gene alterations affect the drug sensitivity of cancer to chemotherapy and targeted therapies and are potential biomarkers for drug identification. Therefore, further exploration was undertaken to investigate the relationship between the mRNA expression levels of the lactylation gene and drug IC50. Analysis of the GDSC database revealed a significant association between increased expression of HAGH and chemoresistance to various GDSC small molecules (Fig. 13A). In addition, high expression of SIRT2, GLO1, HDAC3, HDAC1, SIRT1, HDAC2, EP300, and CREBBP correlated with trametinib resistance. However, high expression of SIRT3, HDAC8, HDAC3, HDAC1, SIRT1, HDAC2, EP300, and CREBBP predicted better sensitivity to multiple GDSC small molecules. The CTRP database further demonstrated that elevated levels of SIRT3, HDAC8, HDAC3, HDAC1, SIRT1, HDAC2, EP300, and CREBBP were indicative of heightened susceptibility to small molecule drugs, whereas increased expression of HAGH was associated with diminished

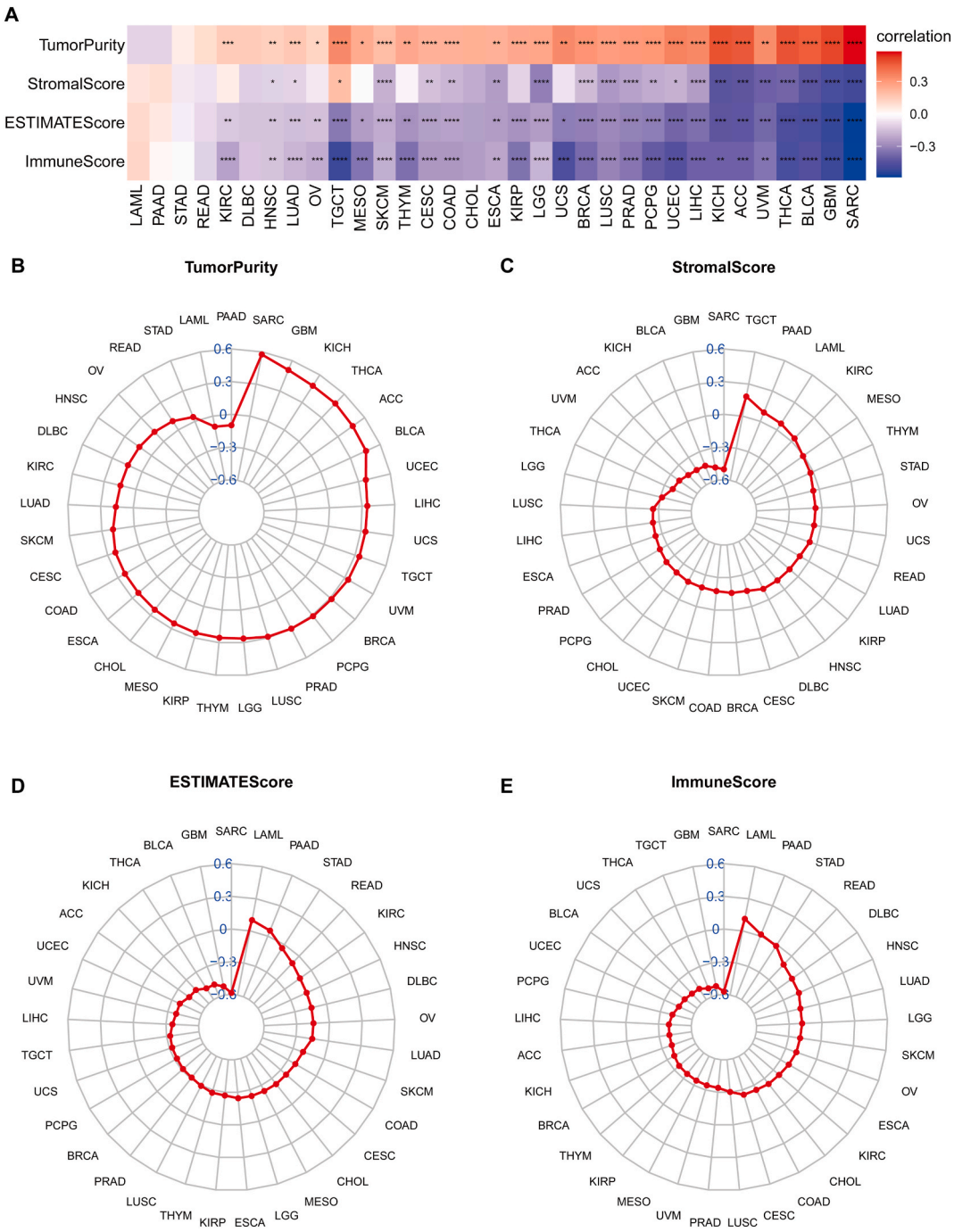

Fig. 8. Correlation between lactylation scores and tumor microenvironment (TME). (A) Heat map of correlation between lactylation and immune score, stromal score, estimate score, and tumor purity in pan-cancer, where red depicts positive correlation and blue depicts negative correlation. Radar map of the correlation between lactylation and (B) tumor purity, (C) stromal score, (D) estimate score and (D) immune score in pan-cancer. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

sensitivity to small molecule drugs (Fig. 13B). In conclusion, abnormal expression of lactylation genes may mediate resistance to chemotherapy and targeted drug therapy.

3.10. Validation of the expression of lactylation genes

Next, we performed RNA-sequencing on the breast cancer tissues and glioma cells to further identify the expression levels of

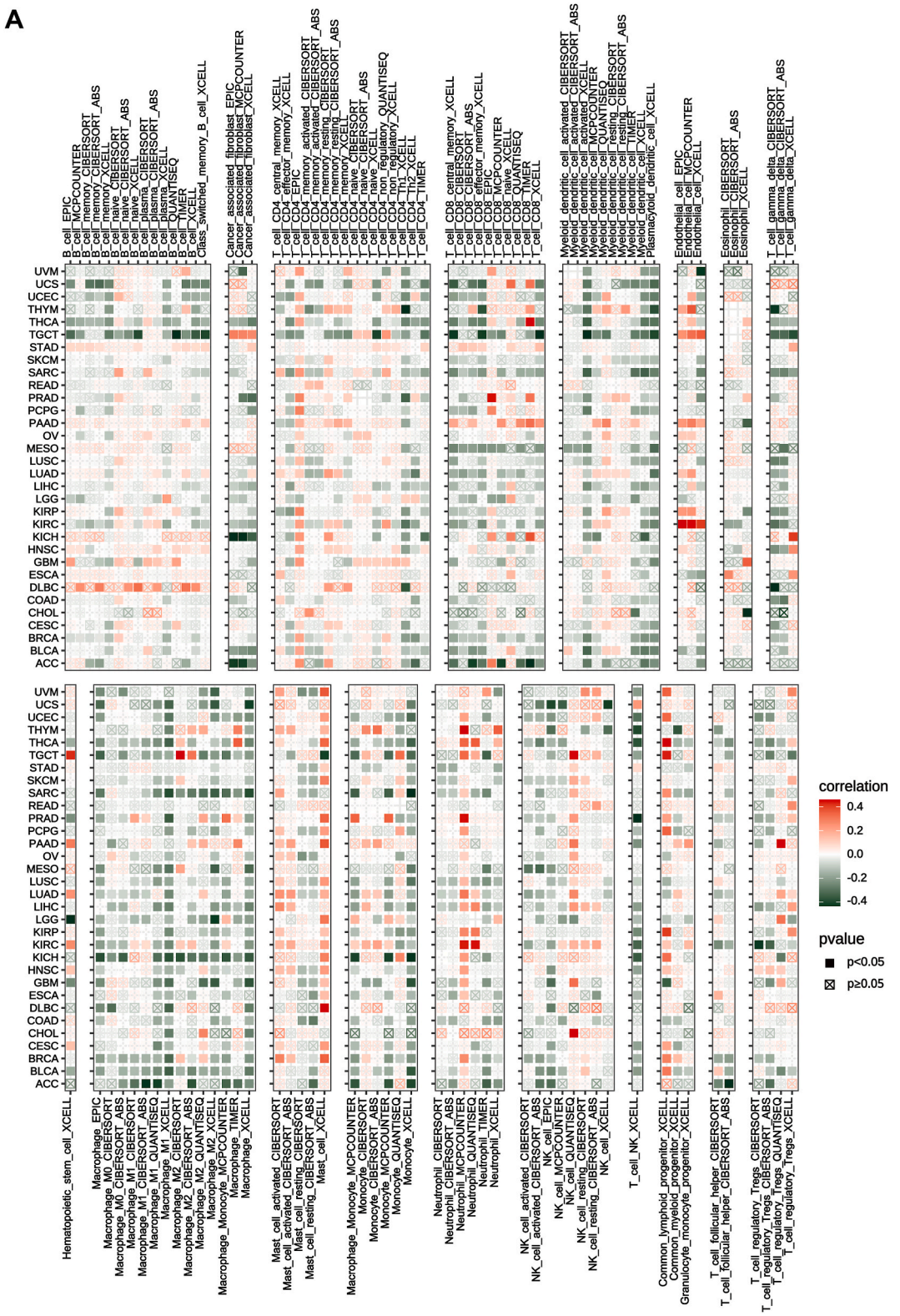

Fig. 9. (A) Correlation of lactylation scores with immune cell infiltration based on TIMER2 database, where red depicts positive correlation and green depicts negative correlation. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

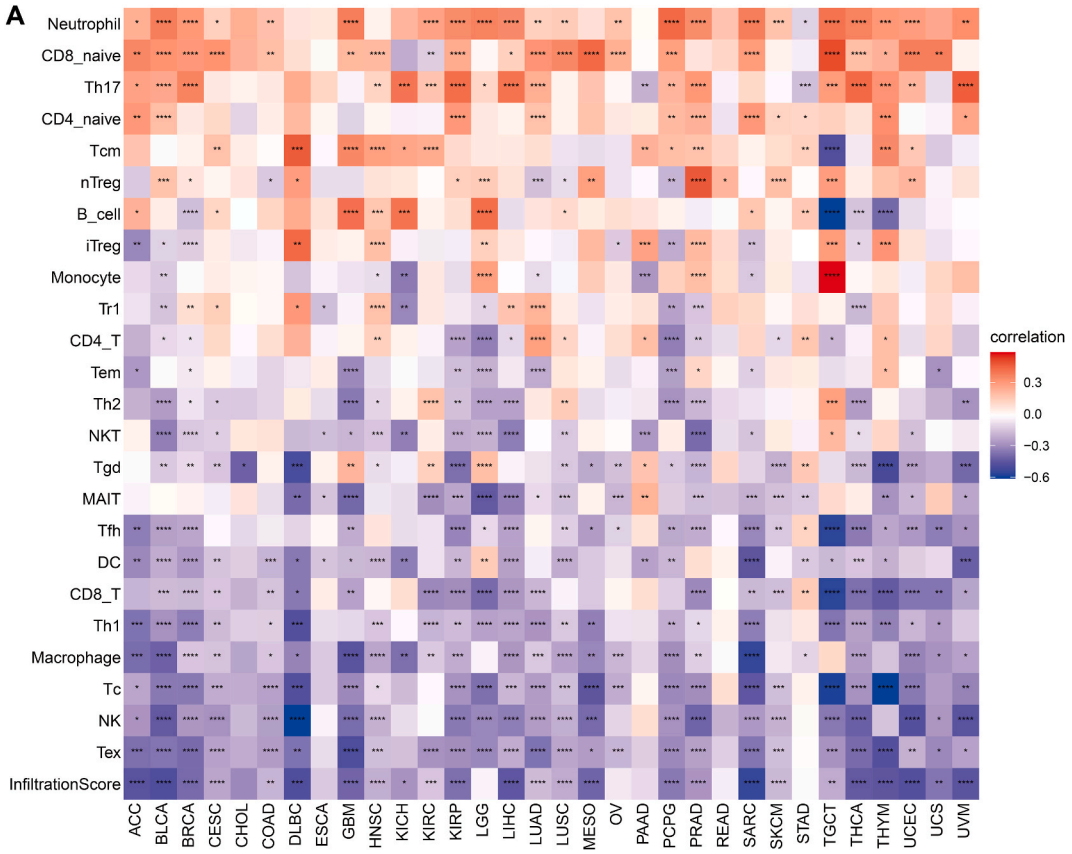

Fig. 10. (A) Correlation of lactylation scores with immune cell infiltration based on ImmuCellAI database, where red depicts positive correlation and blue depicts negative correlation. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

lactylation genes. HDAC2 were remarkably up-regulated in the breast cancer tissues in comparison with the adjacent tissues while SIRT1, SIRT2, SIRT3, and CREBBP were down-regulated in breast cancer tissues (Fig. 14A). In addition, HDAC2, HDAC8, HDAC1, EP300, CREBBP, SIRT1, SIRT2, HAGH and SIRT3 were remarkably up-regulated in U251 cells in comparison with the HMO6 cells while GLO1 and HDAC3 were down-regulated in the U251 cells (Supplementary Fig. 6). Immunohistochemistry for lactylation genes in glioma and normal tissues yielded similar results (Supplementary Figs. 7A–K). Therefore, lactylation genes were differentially expressed between tumor and normal tissues.

4. Discussion

The significance of lactylation modification as an epigenetic modification modality has been well-established [37]. Investigating its role in cancer is of great importance for understanding cancer development and developing therapeutic strategies centered around lactylation modifications. Through the analysis of TCGA multi-omics data, a comprehensive characterization of lactylation genes in 33 different cancer types was conducted. Furthermore, lactylation scores were calculated for each cancer type, revealing a strong association between lactylation scores and tumor immune cell infiltration. Additionally, it was found that lactylation scores could serve as a predictive indicator of patient response to immunotherapy. This analysis provides a comprehensive picture of the regulation of lactylation genes in cancer.

Previous data indicated an increase in the transcript and protein levels of EP300 and HDAC1-3 in different malignancies, with hepatocellular carcinomas (HCC) showing particularly elevated expression [38]. Our results demonstrated that lactylation genes, especially GLO1, HDAC2, HDAC8, and HDAC1, were overexpressed in most tumor tissues, indicating the important function of dysregulation of lactylation genes in cancer development [39–41]. Although HDAC1, HDAC2, HDAC3, EP300, SIRT1, SIRT2, and SIRT3 are the most frequently studied lactylation genes in cancer, it was also found that some other genes, such as GLO1, HDAC8, CREBBP, and HGH, exhibit not only high expression levels in most tumors but also serve as multiple tumor prognostic risk factors [42]. GLO1 has previously been shown to be proportional to the metastatic potential of SN12C human renal cell carcinoma cells [43]. The inhibition of the EP300/CREBBP bromodomain disrupts critical oncogenic transcriptional programs regulated by transcription factors including MYC, IRF4, GATA1, and AR [44]. Furthermore, HDAC8 inhibition reduced tumor volume in a mouse glioma model, which is

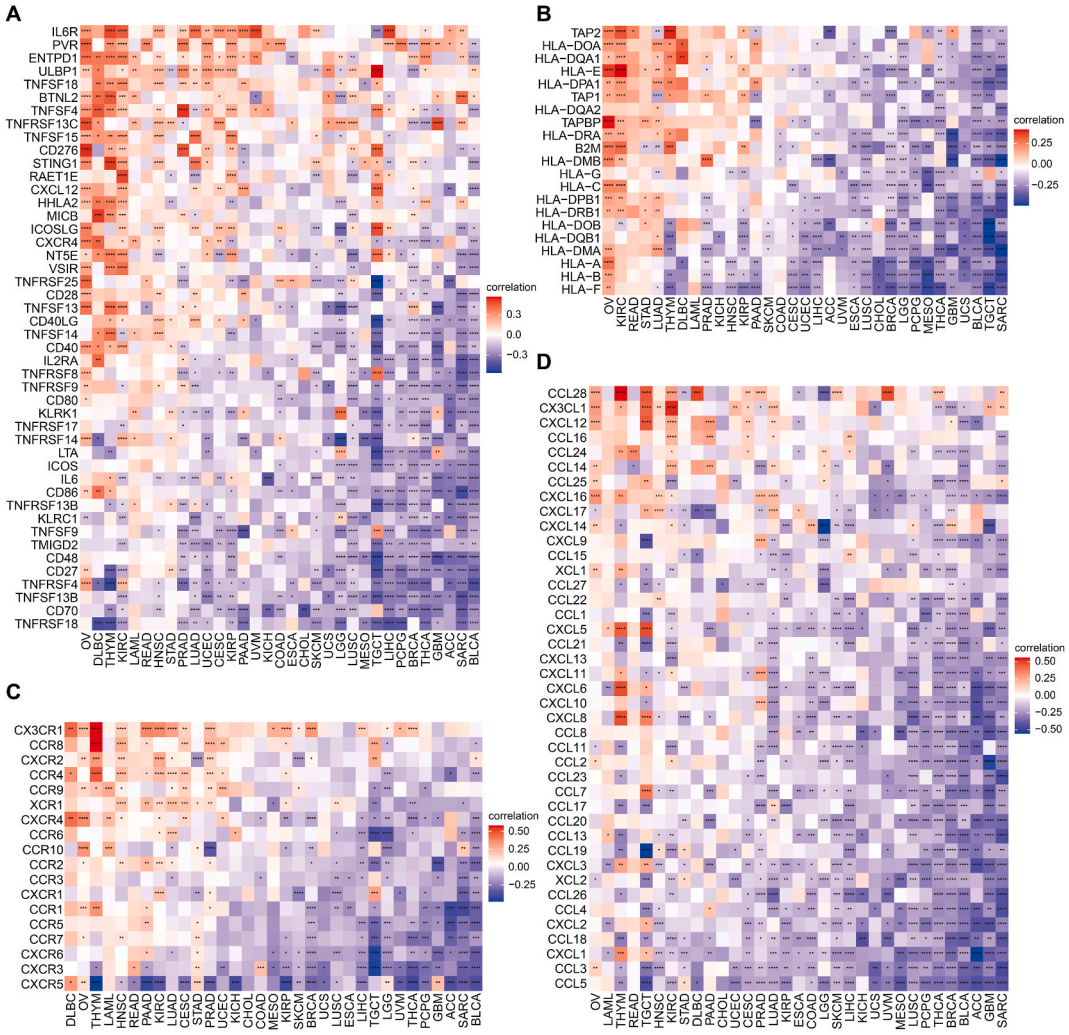

Fig. 11. Correlation of lactylation scores with immunomodulatory genes.
The heat map shows the correlation between the lactylation scores and (A) immune activation genes, (B) MHC genes, (C) chemokine receptors and (D) chemokines in various tumors, where red and blue depict positive and negative correlations, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

consistent with the outcomes of this research [45]. Although the results of this research revealed that multiple lactylation genes are tumor suppressors in KIRC, Yang et al. demonstrated that aberrant histone lactylation triggered by inactive VHL promotes KIRC progression by activating PDGFRβ transcription [46]. The conflicting outcomes may be attributed to the intricate nature of lactylation modification, which involves multiple genes and encompasses a complex multistep process. Additionally, the results of lactylation modification are more prominent than the effects on regulating lactylation gene expression, and the underlying mechanisms deserve further in-depth investigation. In conclusion, lactylation genes demonstrate varying levels of expression across pan-cancer cases and possess unique prognostic significance in diverse cancer types.

The study explored several genomic alterations that may affect lactylation gene expression, including SNV, CNV, and promoter methylation. Notably, CREBBP and EP300 emerged as the most frequently mutated genes across different cancer types. Additionally, prior research supports the findings of the current study. CREBBP and EP300 are frequently mutated in cutaneous squamous cell carcinomas and lymphomas, and these mutation types can promote tumorigenicity compared to wild-type CREBBP and EP300 proteins [47,48]. CREBBP/EP300 mutations promoted tumor progression in diffuse large B-cell lymphoma through altering tumor-associated macrophage polarization via FBXW7-NOTCH-CCL2/CSF1 axis [48]. Efforts to target CREBBP and EP300 mutant sites are increasing, and CREBBP mutation-related targeted agents have been approved for clinical trials [49]. They may be potential targets for developing specific drugs targeting lactylation modifications. CNV analysis revealed that lactylation genes exhibit a non-cancerous type--dependent amplification or deletion pattern. This research hypothesized that in some cases, their co-amplification or co-deletion may lead to dysregulation of lactylation to promote tumor progression. Additionally, the epigenetic analysis yielded novel insights into the contrasting methylation patterns of lactylation genes in tumor and normal tissues. Specifically, KIRP, UCEC, and BRCA exhibited

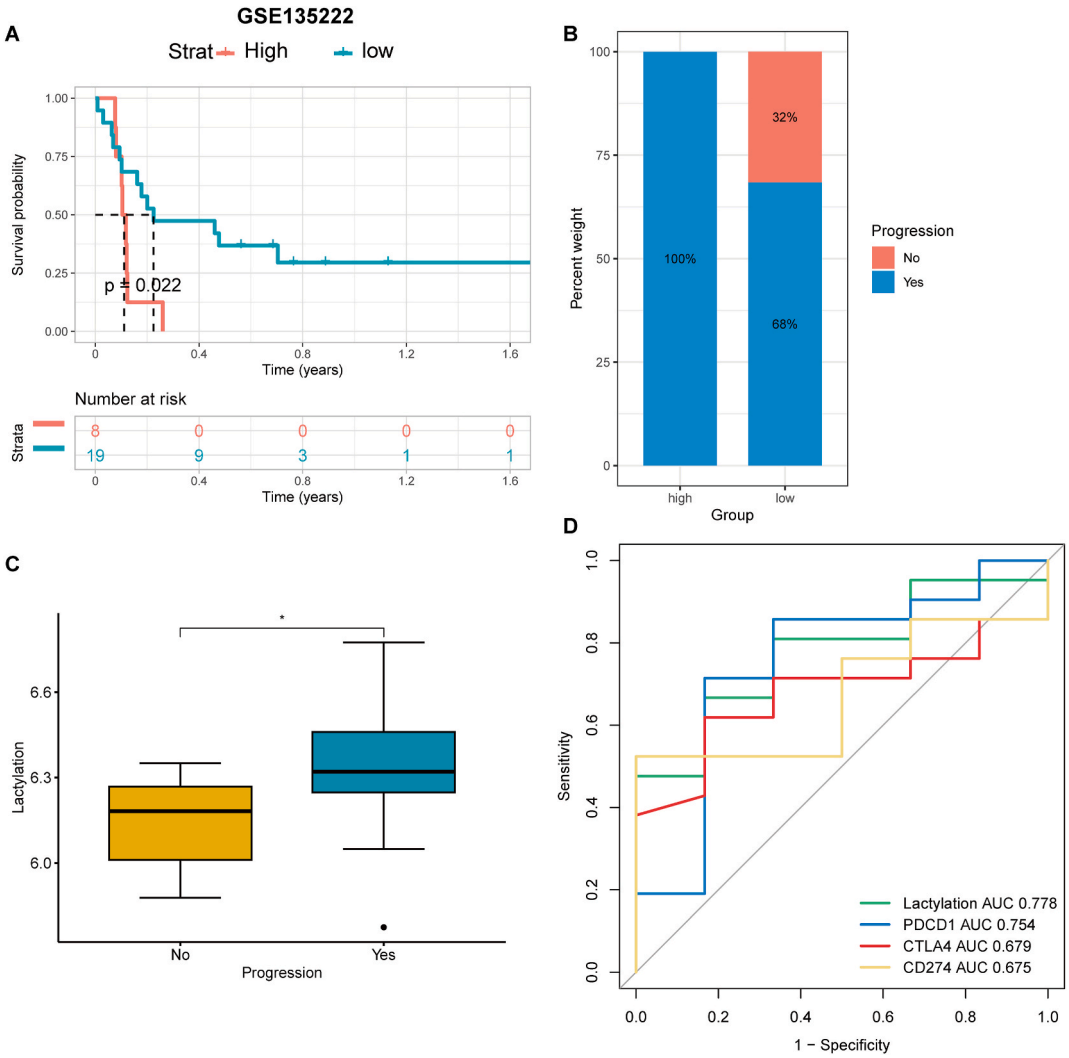

Fig. 12. Predictive efficiency of lactylation scores in the GSE135222 immunotherapy cohort. (A) Kaplan-Meier curves for lactylation scores in the GSE135222 cohort. (B) The proportion of patients with progressive disease in different lactylation score groups. (C) Differences in lactylation scores between progressive and nonprogressive patients. (D) The ROC curve of lactylation score and immune checkpoints (PDCD1, CTLA4 and CD274) to predict the effect of immunotherapy.

heightened methylation levels, whereas HNSC and KIRC displayed diminished methylation levels in lactylation genes. However, more experimental evidence is needed to elucidate the genetic and epigenetic roles of lactylation genes in cancer development and prognosis.

This research used the widely accepted ssGSEA algorithm to assess the lactylation profile from 33 cancer types. Interestingly, lactylation scores were similar between tumor types, suggesting a similar lactylation environment between different tumors. The prognostic impact of lactylation scores varied by tumor type, with both Cox analysis and K-M analysis showing that lactylation scores were protective factors for several tumors, including KIRC, ACC, READ, LGG, and UVM. Prior research has demonstrated that lactylation modification is linked to tumor progression in melanoma and colorectal cancer [23,50]. The conflicting results in rectal cancer may stem from the number of markers analyzed. Due to study limitations, only one lactylated protein was analyzed in these studies, and perhaps this narrow view is insufficient to provide a holistic perspective. Instead, in this study, the assessment of lactylation enrichment was conducted based on 11 major lactylation-related genes, aiming to derive more accurate conclusions. Furthermore, the varying prognostic impacts of distinct tumor lactylation scores may be attributed to the fluctuating patterns of lactylation modifications, diverse modification sites, interactions with other epigenetic modifications, sample size, individual variations, and other factors that necessitate further investigation and elucidation. In the cases of CHOL, DLBC, LAML, and OV, high lactylation scores have been linked to unfavorable prognoses based on the K-M analysis. Targeted therapies designed to specifically address lactylation modifications could hold promise as potential therapeutic interventions. However, to fully assess their efficacy and safety, further research and clinical trials are imperative.

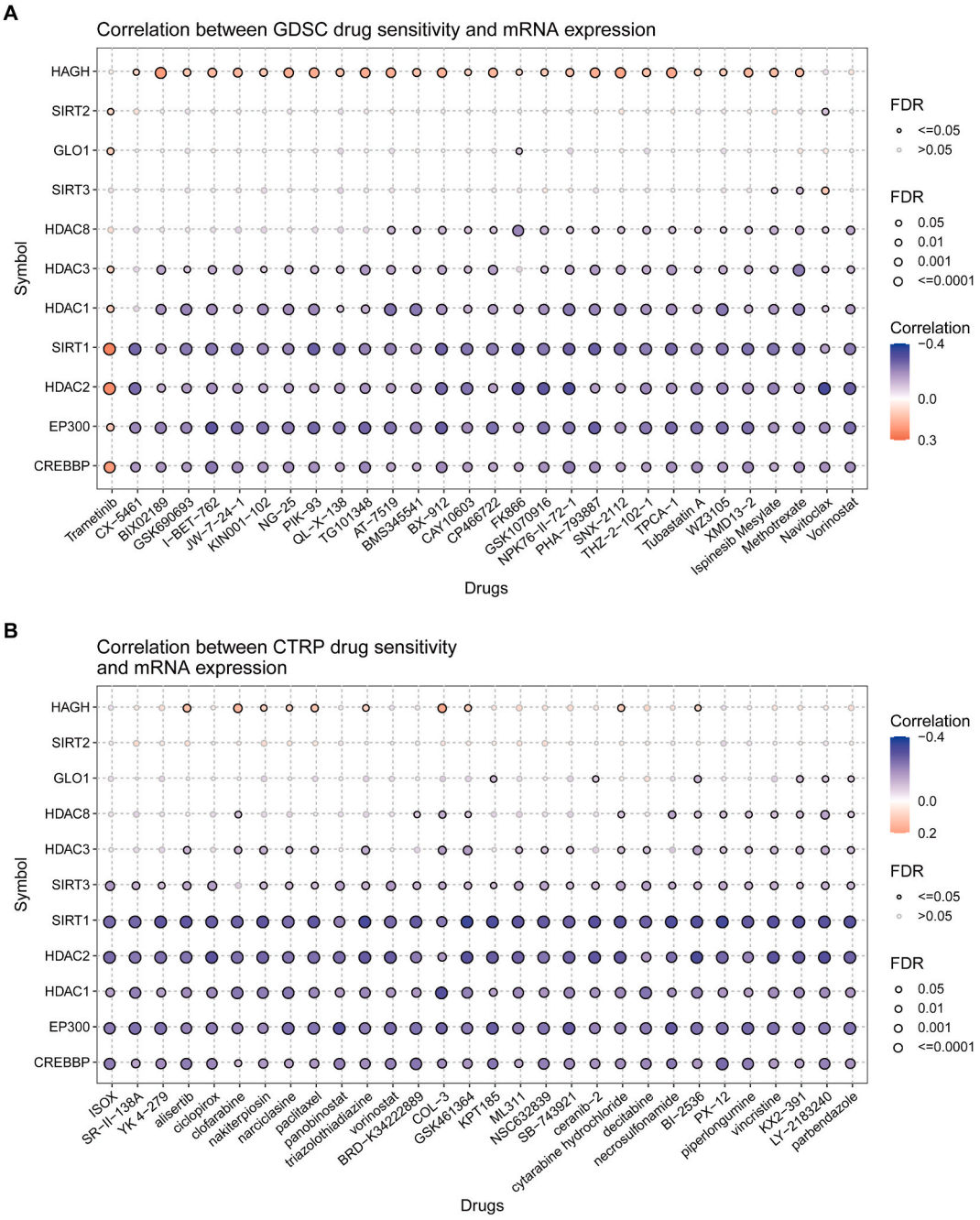

Fig. 13. Correlation of lactylation genes with drug sensitivity. (A) Correlation between IC50 values of top 30 GDSC drugs and lactylation gene expression in pan-cancer. (B) Correlation between IC50 values of top 30 CTRP drugs and lactylation gene expression in pan-cancer. Red bubbles depict positive correlations, and blue bubbles depict negative correlations, with darker colors suggesting higher correlations. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

The application of GSVA revealed a significant correlation between lactylation scores and various cancer pathways, with a particular emphasis on pathways related to immunoregulation. A mounting body of evidence supports the notion that lactylation plays a crucial role in shaping the TME [11]. Lactate-induced histone lactylation is closely associated with macrophage M2-type polarization [37]. In addition, the upregulation of METTL3 driven by lactylation contributes to the immunosuppressive capabilities of myeloid cells infiltrating the tumor [23]. However, these studies have focused on analyzing one class of immune cells, and few studies have evaluated the role of lactylation in TME. Therefore, the characteristics of TME were assessed from the perspective of lactylation scores. The findings demonstrated that the lactylation score was notably and negatively associated with stromal score, estimate score, and

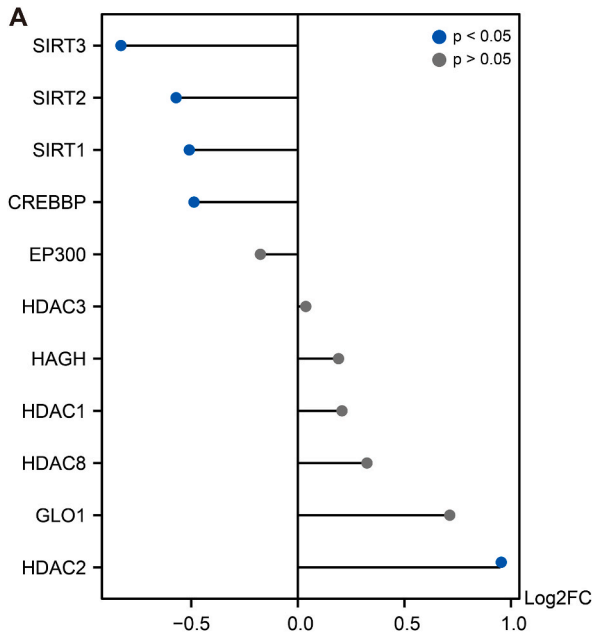

Fig. 14. (A) The expression levels of lactylation genes in 26 cases of breast cancer and adjacent tissues were detected by RNA-sequencing.

immune score, suggesting that a high lactylation score is closely associated with cold TME. In addition, based on publicly available immune cell infiltration data, it was found that the lactylation score was significantly and negatively associated with $CD8^+$ T, γδ T, NKT, and NK cells. lactylation scores were also negatively correlated with most immunomodulatory genes in various tumors. The results of this study suggest that high lactylation scores promote the generation of an immunodesert TME, suggesting that patients with high lactylation scores may not respond well to immunotherapy [51]. Possible intervention strategies include improving immune cell infiltration and function, as well as restoring immune responses through modulation of the TME.

ICB significantly improves survival in patients with advanced cancer [52–54]. However, in most cancer types, only about one-third of patients respond to immunotherapy [55]. Therefore, the development of biological markers that accurately predict the sensitivity of ICB is imminent. In a study conducted by Yang et al. it was determined that lactylation levels can be used to assess the therapeutic response to ICB in patients with gastric cancer [56]. In our analysis of immunotherapy data, we observed that individuals with higher lactylation scores who underwent immunotherapy exhibited poorer treatment outcomes and prognosis. Notably, the lactylation score profile gave an AUC value of 0.778 on the ROC curve for predicting the effect of immunotherapy, which was significantly better than the immune checkpoint. Hence, indicating that it is an excellent predictive biomarker for ICB. Moreover, lactylation scores carry significant clinical implications for monitoring treatment response and evaluating prognosis. Further studies are required to validate their accuracy and offer guidance for their clinical application. Bicarbonate administration to inhibit lactate-induced acidic environment has been shown to inhibit melanoma growth, increase $CD8^+$ T cell infiltration, and improve sensitivity to ICB therapy in B16 mice [23]. In addition, Gao et al. found that lactate depletion produced immunoreactive TME that effectively enhanced the antitumor effect of anti-PDL1 therapy [57]. The results of this research support the proposal that anti-lactylation modifications can be considered a therapeutic strategy for putative enhancers of ICB therapy. This combination therapy exhibits the potential to augment the efficacy of immunotherapy and surmount the resistance observed in the cold TME. Therefore, exploring combination therapy is a necessary and feasible research direction.

However, it is important to acknowledge certain limitations within this study. Primarily, due to the nascent stage of lactylation research, only a subset of genes influencing lactylation were incorporated in this investigation. Secondly, all findings are predicated upon published data. Given the limitations of extant clinical data, there exists an exigent necessity for additional prospective data to corroborate the attributes of lactylation genes and lactylation scores. Third, due to insufficient data on immunotherapy, this study explored the prediction of the lactylation score on immunotherapy response in only one cancer cohort. More studies on immunotherapy data are urgently needed. Overall, this study provides a new comprehensive perspective in exploring the dysregulation of lactylation genes in cancer. In addition, the lactylation score is closely associated with cold TME in pan-cancer. The lactylation score is a potential biological marker for the treatment of ICIs in oncology patients.

5. Conclusion

This research has provided a comprehensive overview of lactylation genes in 33 TCGA malignancies in terms of transcriptomics, genomics, and drug sensitivity and provided an indispensable reference for studying the role of lactylation in TME and immunotherapy. Our findings suggest that lactylation genes, specifically GLO1, HDAC2, HDAC8, and HDAC1, are significantly upregulated in

tumor tissues and hold promise as therapeutic targets. Moreover, the lactylation score demonstrates strong potential as a biomarker for predicting patient prognosis and immunotherapy efficacy. This systematic study of lactylation will provide novel insight for understanding aberrant lactylation in cancer and contribute to developing lactylation-targeted therapies.

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Data availability statement

All data included in this study are available by contacting the corresponding authors.

Ethics approval and consent to participate

The studies involving human participants were reviewed and approved by Ethics Committees of the First Affiliated Hospital of Wenzhou Medical University. The patients provided their written informed consent to participate in this study.

Patient consent for publication

Not applicable.

CRediT authorship contribution statement

Zhixuan Wu: Writing – original draft, Investigation. **Haodong Wu:** Writing – original draft, Investigation. **Yinwei Dai:** Writing – original draft, Investigation. **Ziqiong Wang:** Writing – review & editing, Methodology. **Hui Han:** Writing – review & editing, Methodology. **Yanyan Shen:** Writing – review & editing, Project administration. **Rongrong Zhang:** Writing – review & editing, Project administration. **Xiaowu Wang:** Writing – review & editing, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e27465.

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