

Research Article

Lactate-Driven Histone Lactylation Weakens B Cell Immunity in Tertiary Lymphoid Structures

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Abstract

The function of tertiary lymphoid structures (TLS), which are important locations in the tumour microenvironment that promote anti-tumor humoral immunity, is frequently depleted in advanced tumours, and the underlying mechanisms are yet unknown. Through histone lactylation, the tumour metabolite lactate modifies the epigenetic status of B cells in TLS, thereby compromising their immunological activity. We used single-cell RNA sequencing, chromatin accessibility studies, and microscopic dissection of mature TLS from clinical non-small cell lung cancer tissues and animal tumour models. Lactate concentrations within TLS were determined using targeted quantitative metabolomics, and the genome-wide H3K18 lactylation signature in B cells was mapped using CUT&Tag technology. The functional impact of histone lactylation on B cell antibody generation and anti-tumor efficacy was assessed utilising adoptive transfer procedures, B cell receptor lineage tracing, and immunofluorescence in a mouse model with B cell-specific deletion of lactate dehydrogenase A. High lactate concentrations in the tumour microenvironment cause TLS-B cells to abnormally concentrate H3K18 lactylation in immunoglobulin class switch recombination sites and promoters of genes linked to somatic hypermutation. IgG2b/c class switching and affinity maturation are prevented by this epigenetic remodelling, which directly reduces the production of activation-induced cytidine deaminase and essential transcription factors. According to functional studies, mature germinal center responses in TLS can be restored by suppressing lactylation reading proteins or preventing lactate metabolism in B cells. This greatly improves antibody-dependent tumour killing and reverses resistance to immune checkpoint blockade therapy. The “lactate-histone lactylation” axis is identified in this study as a novel metabolic checkpoint for B cell immunity within TLS. This discovery reveals a pathogenic pathway by which the immunosuppressive microenvironment takes control of humoral

immunity at the epigenetic level and offers specific targets for the development of combination immunotherapies aimed at restoring TLS functionality.

Keywords

Tertiary lymphoid structures, Histone lactylation, B cell immunity, Tumor microenvironment, Immune metabolism

1. Introduction

The majority of patients with solid tumours still struggle to achieve long-lasting responses, despite the fact that immune checkpoint blockade (ICB), a kind of cancer immunotherapy, has altered the clinical treatment landscape. Recent clinical research has shown that mature tertiary lymphoid structures (TLS) in tumour tissue are an independent positive predictor of ICB efficacy and patient survival. TLS are lymphoid aggregates that form ectopically in tumour or chronic inflammatory sites. They have well-organised T cell and B cell zones. High-affinity tumor-specific antibodies and effective anti-tumor humoral immunity can be produced as a result of their promotion of plasma cell development, germinal center (GC) responses, and in situ antigen presentation. However, when tumours grow, even mature TLS often show poor affinity maturation, delayed antibody class flipping, and B cell zone depletion, which results in a “silent” local humoral immune response. Both the metabolic microenvironment’s role in this process and the molecular switches that cause B cell immune function in TLS to shift from “active” to “exhausted” remain poorly understood.

One of the characteristics of malignant transformation is metabolic reprogramming of the tumour microenvironment (TME). Large volumes of lactate are generated and exported to the stroma as a result of tumour cells’ tendency to favour glycolysis even in aerobic environments in order to fulfil the demands of unrestricted growth (the Warburg effect). Tens of millimoles of lactate can be found in the TME, which is significantly more than what is found in healthy tissues. Long thought to be merely a metabolic waste product, lactate is now a multidimensional immunological signalling molecule in addition to an energy carrier and a factor in acidic stress, according to research conducted over the past ten years. Tumor-associated macrophages can be polarised toward an immunosuppressive M2-like phenotype, regulatory T cell differentiation can be encouraged, and T cell and NK cell cytotoxic activities can be directly inhibited. More remarkably, Zhang and associates discovered in 2019 that lactate can act as a direct substrate to promote lactylation of histone lysine residues, a novel post-translational modification that directly connects chromatin structure and gene transcription to cellular metabolic conditions. Histone lactylation is implicated in the regulation of oncogene expression in different tumour cells and has been demonstrated to minimise inflammatory damage in macrophages during the late phase of M1 polarisation by activating repair gene expression. However,

there is still a critical knowledge gap that has to be addressed: if histone lactylation influences B cell fate decisions, particularly in tumor-infiltrating TLS in this locally severe metabolic milieu, and whether it reduces B cell effector function epigenetically.

Class switch recombination (CSR) and somatic hypermutation (SHM) of immunoglobulins are essential mechanisms for the production of high-affinity protective antibodies in germinal center B cells. A gene regulatory network consisting of important transcription factors like as Bcl-6, Irf4, and BATF controls both of these processes, which are absolutely dependent on the precise, transient expression of activation-induced cytidine deaminase (AID). Antibody affinity maturation and the variety of effector activities can be compromised by any disruption in this network or its upstream epigenetic environment. Notably, complex regulatory elements are abundant in genomic locations encoding AID, IgG2b/c constant sections, and germinal center maintenance genes, and their chromatin accessibility is highly sensitive to signals from the microenvironment. Based on this, we speculate that persistently high levels of lactate in the TME may enter the TLS-GC regions, be absorbed by B cells, and transform into substrates for epigenetic modifications. These modifications may then “hijack” the gene expression programs required for antibody maturation through histone lactylation, thereby constituting a metabolic checkpoint that has not yet been identified.

The involvement of lactate-driven histone lactylation in TLS-B cell immunological fatigue is the main topic of this investigation. In order to map the lactylation profiles of B cells within TLS and their relationship to functional exhaustion, identify the molecular mechanism by which lactate directly suppresses AID expression and antibody class switching through histone lactylation, and determine whether blocking lactate metabolism or inhibiting lactylation readers can restore TLS germinal center responses and boost anti-tumor humoral immunity, we combined clinical non-small cell lung cancer samples, multiplex immunofluorescence visualisation, microdissection with multi-omics analysis, and B cell conditional knockout mouse models. These results may demonstrate the importance of the “metabolism-epigenetic” axis in the control of TLS function and provide novel approaches based on TLS function restoration to combat ICB resistance.

2. Literature Review

2.1. Tertiary lymphoid structures and tumor B cell immunity

Tumours and areas with persistent inflammation can develop lymphoid aggregates known as tertiary lymphoid structures (TLS). Their cellular composition and spatial arrangement, which include networks of follicular dendritic cells, zones of CD4⁺ and CD8⁺ T cells, and CD20⁺ B cell follicles, are strikingly comparable to those of secondary lymphoid organs. Even functioning germinal centers (GC) can emerge in certain mature TLS (Dieu-Nosjean et al., 2008; Fridman et al., 2022). The density of mature TLSs within tumours is strongly positively correlated with long-term patient survival in

non-small cell lung cancer, as demonstrated by Dieu-Nosjean and colleagues (2008). Since then, a number of groundbreaking research have solidified TLS's status as a "on-site factory" for anti-tumor immunity: Germain and colleagues (2014) discovered that B cells in TLS can produce tumor-specific IgG, which can cause antibody-dependent cellular cytotoxicity; TLS and their internal B lymphocytes are among the best independent predictors of how solid tumours like sarcoma and melanoma react to immune checkpoint blockade (ICB), according to three consecutive studies published in Nature in 2020 (Cabrita et al., 2020; Helmink et al., 2020; Petitprez et al., 2020). The GC-B cell zones frequently exhibit traits of "functional silence," such as blocked class-switch recombination (CSR), defects in affinity maturation, and exhaustion in plasma cell differentiation, even in tumours rich in TLS, according to clinical pathological analysis. The molecular picture of what microenvironmental signals cause TLS B cells to transition from "activation" to "exhaustion" is currently unclear.

2.2. Tumor microenvironment lactate metabolism and immune suppression

Lactate concentrations in the tumour stroma can reach 20-40 mM, which is significantly greater than in normal tissues, due to the heightened Warburg effect that tumour cells typically exhibit with aerobic glycolysis (Warburg, 1956). Research conducted in the last ten years has fundamentally altered the long-held belief that lactic acid is a waste product of metabolism. Colegio and colleagues (2014) discovered that via stabilising HIF-1 α , tumor-derived lactic acid can directly cause tumor-associated macrophages (TAMs) to polarise toward an immunosuppressive M2-like phenotype. High lactic acid concentrations have been shown by Brand and others (2016) to inhibit the cytotoxic activities of T cells and NK cells, which lowers IFN- γ production and weakens tumour immune surveillance. Foxp3⁺ regulatory T cells (Tregs) can sustain significant immunosuppressive activities in the tumour microenvironment (TME) by physiologically adapting to use lactate as an alternative energy source in situations with high lactate and low glucose, as demonstrated by Angelin and colleagues (2017). In their analysis, Wang and colleagues (2021) provided a methodical overview of the several ways that lactate modifies the anti-tumor immune network, including G protein-coupled receptor signalling, post-translational alterations, and epigenetic regulation. Lactic acid has well-established suppressive effects on innate immunity and T cell immunity, but less is known about how it directly regulates B cell lineages, particularly the fate of GC-B cells within TLS.

2.3. Histone lactylation modification: From discovery to function

Lactate can behave as a completely new substrate for epigenetic alteration, directly promoting lactylation on histone lysine residues, according to a 2019 study by Zhang and colleagues (2019) published in Nature. Acyltransferases like as p300 catalyse this change, which occurs independently of acetylation. Their research demonstrated that lactate accumulated in M1 macrophages during the late stage of metabolic remodelling can initiate repair-related gene expression (such as Arg1) through

H3K18 lactylation, thereby reducing inflammation. This essentially highlights lactylation as a direct link between chromatin and metabolism. Subsequently, Moreno-Yruela and colleagues (2022) discovered that class I histone deacetylases (HDAC1-3) exhibit histone delactylase activity, indicating that a dynamic “write-erase” balance finely regulates cellular lactylation levels. Yu and colleagues (2021) discovered that uveal melanoma can be caused by histone lactylation in the field of tumours by encouraging the expression of the m-Numerous studies have also connected aberrant lactylation alteration to immune evasion, tumour cell proliferation, and stemness maintenance in the reader protein YTHDF2. In their review, Dai and colleagues (2022) noted that lactylation may affect gene regulation in different immune cell subtypes in quite distinct ways. Nevertheless, no research has yet shown the biological roles and modification patterns of histone lactylation in B cells, particularly those that have undergone the GC response.

2.4. Metabolic and epigenetic regulation of B cell antibody responses

Significant shifts in metabolic needs are involved in somatic hypermutation (SHM), CSR, and B cell activation. In order to support rapid proliferation and antibody secretion, GC B cells must precisely balance glycolysis and oxidative phosphorylation, as reviewed by Boothby and Rickert (Jellusova & Rickert, 2016). Jellusova goes on to describe the crucial role of the PI3K-Akt-mTOR pathway in integrating metabolism and transcription programs in determining B cell fate (Ersching et al., 2017). At the epigenetic level, activation-induced cytidine deaminase (AID) is the only enzyme that can initiate and carry out CSR in the immunoglobulin heavy chain constant region gene locus. AID targets the transcriptionally active S regions for deamination, and modifications such as histone acetylation and H3K4me3 precisely control the chromatin accessibility of the S regions (Bolland et al., 2016; Chen & Wang, 2019). Daniel and colleagues verified that the epigenetic regulator PTIP maintains H3K4me3 modifications in the S region to ensure normal CSR; Pavri and colleagues (2010) found that AID targeting is highly sensitive to the local chromatin state and depends on RNA polymerase II pausing and interacting with Spt5. While it is known that lactate can increase global acetylation levels by inhibiting HDAC activity, it is still largely unknown if lactate can directly alter the chromatin landscape of CSR/SHM-related gene loci through this novel epigenetic pathway of histone lactylation and thereby affect antibody affinity maturation.

2.5. Cutting-edge technologies provide an opportunity to explore the metabolic and epigenetic regulation of TLS-B cells

The fast advancement of single-cell omics technology over the last ten years has made it possible to analyse the functional states and heterogeneity of uncommon cell populations with previously unheard-of detail. Single-cell chromatin accessibility sequencing (scATAC-seq) can more clearly show the activation of cis-regulatory elements in individual cells (Buenrostro et al., 2015), while single-cell

RNA sequencing (scRNA-seq) has been extensively utilised to map tumor-infiltrating immune cells (Stubbington et al., 2017). It's particularly noteworthy that the bottleneck of standard ChIP-seq, which requires a large number of beginning cells, can be overcome by mapping genome-wide histone modifications in a small number of cells thanks to the CUT&Tag technology created by Kaya-Okur and others (2019). These state-of-the-art instruments can now be used in conjunction with laser capture microdissection technology to accurately sample intact TLS structures within tumour tissues and sort small quantities of GC-B cells for multi-omics analysis. This provides a methodological basis for deciphering B cell lactylation profiles in the actual metabolic environment *in vivo*.

2.6. Limitations of existing research and this study's contributions

According to the research background mentioned above, the current knowledge system has the following major gaps: we don't know anything about the presence and regulatory role of histone lactylation in lymphocytes, particularly GC-B cells; it's unclear whether lactate can directly affect AID expression and the CSR/SHM process through histone lactylation as an epigenetic pathway; and there's no direct evidence demonstrating whether B cell dysfunction in tumour TLS is driven by the local metabolic environment. In their study of B cells and antibody repertoires in the TME, Sharonov and colleagues highlighted the role of metabolism and epigenetics in tumour B cell fatigue; however, genuine experimental studies are still lacking (Laumont et al., 2022).

The 'lactate-histone lactylation' axis is introduced into the realm of TLS-B cell immune function regulation for the first time in this work, which expands on the unresolved concerns discussed above. This study intends to systematically map the histone lactylation landscape of TLS-GC-B cells in a lactate-rich environment using a multi-omics system that combines microdissection-TLS with scRNA-seq, scATAC-seq, and CUT&Tag. It also seeks to identify key sites where lactylation modifications regulate AID expression and antibody class switching and validate their functional roles in anti-tumor humoral immunity using B cell-specific *Ldha* knockout mice and lactylation reader inhibition techniques. It is anticipated that this study will close the gap between immunometabolism and B cell epigenetic regulation and establish histone lactylation as a theoretical foundation for novel targets to restore TLS function.

3. Research Methods

From 78 cases of non-small cell lung cancer (NSCLC), we obtained tumour tissues and matched surrounding normal tissues (at least 5 cm from the tumour margin). Pathologically verified lung adenocarcinoma or squamous cell carcinoma; absence of chemotherapy, radiation, or immunotherapy prior to surgery; and multiplex immunofluorescence confirmation of CD20⁺ B cell clusters in paraffin-embedded tissue sections are the inclusion criteria. Other malignant tumours or autoimmune

disorders are exclusion criteria. In less than an hour, freshly removed tissues were treated in cold RPMI-1640 media.

By crossing *Ldha*(fl/fl) mice (Jackson Laboratory, B6;129S-*Ldha*(tm1c(EUCOMM)Wtsi)) with *Cd19-Cre* transgenic mice (B6.Cg-Tg(*Cd19-cre*)1Cgn/J), B cell-specific *Ldha* knockout mice were produced. B-*Ldha*-KO, also known as *Cd19-Cre⁺*. *Ldha*, the littermate (fl/fl) Mice with *Cd19-Cre⁻* were employed as controls. Each mouse had free access to food and water and was housed in an SPF environment with a 12-hour light/dark cycle.

Lewis lung cancer cells generated from mice Resuspended in 100 μ L of PBS, LLC-OVA (1×10^6) or MC38-OVA colorectal cancer cells (1×10^6) were subcutaneously injected into the right flank of 6–8 week old B-*Ldha*-KO and littermate control mice, with an equal distribution of males and females. Every three days, the tumour volume was measured using a calliper and the following formula: $\text{volume} = \text{length} \times \text{width}^2 / 2$. When the tumour volume reached 2000 mm³ or ulceration developed, the mice were put to death. On days 7, 10, and 13 following inoculation, mice were injected intraperitoneally with 200 μ g of anti-mouse PD-1 antibody (clone RMP1-14, Bio X Cell) or isotype control. There were eight to ten mice in each group.

Fresh tumour tissues were sliced into 8 μ m frozen slices and implanted in OCT. Following quick HE staining, two pathologists separately indicated the TLS areas. A laser capture microdissection device (Leica LMD6500) was used to extract TLS-enriched regions, which were then gathered into centrifuge tubes filled with lysis buffer. Collagenase D (2 mg/mL) and DNase I (0.1 mg/mL) were used to digest certain TLS tissues for 30 minutes at 37°C. The resulting single-cell suspensions were then filtered through a 70 μ m filter. *CD19⁺GL7⁺CD95⁺* germinal center B cells, *CD19⁺CD38⁺* plasmablasts/plasma cells, and *CD3⁺* T cells were obtained by flow sorting immune cells that had been pre-enriched using *CD45* magnetic beads (Miltenyi Biotec). Sorting purity exceeded 95%.

After deparaffinizing the paraffin sections and performing antigen retrieval (pH 6.0 citrate buffer, 95°C for 20 minutes), the sections were blocked for an hour using 5% donkey serum. The primary antibodies (rabbit anti-human/mouse *CD20*, mouse anti-human/mouse *CD3*, rat anti-human/mouse *CD21⁺*, goat anti-human/mouse *Podoplanin*, rabbit anti-*Ki67*, mouse anti-*Bcl-6*, etc.) were incubated at 4°C for an entire night. After an hour of room temperature incubation, corresponding species Alexa Fluor-conjugated secondary antibodies were counterstained with DAPI. The Vectra Polaris multispectral imaging equipment (Akoya Biosciences) was used to scan the sections, and inForm software was used for spectral unmixing and tissue region segmentation. TLS maturity was evaluated using standards from the literature: mature TLS had *Bcl-6⁺Ki67⁺* germinal centers and *CD21⁺* follicular dendritic cell networks, whereas early TLS do not.

Using ¹³C3-labeled lactate as an internal reference, 50 mg of fresh tumour tissue or microdissected TLS sections were homogenised in 80% methanol that had been previously cooled to

-80°C. Ultra-high-performance liquid chromatography-tandem mass spectrometry was used to analyse the supernatant after it had been vacuum-centrifuged to dryness and reconstituted in 50% acetonitrile. The Xevo TQ-S triple quadrupole mass spectrometer was utilised in conjunction with a Waters ACQUITY UPLC system. Using a gradient elution, the mobile phases A and B were 10 mM ammonium acetate (pH 9.0) and acetonitrile, respectively, in a Waters ACQUITY UPLC BEH Amide column. With the internal standard at 92→45 and the lactate ion pair at 89→43, multiple reaction monitoring mode was employed. A standard curve was used for quantification, and the results are displayed in nmol/mg of tissue.

After sorting CD45⁺ cells from the TLS areas of three NSCLC cases and confirming their >90% viability using trypan blue staining, the cells were placed into the 10x Genomics Chromium Controller platform. The Chromium Single Cell 3' v3.1 kit was used to generate libraries. At a depth of roughly 50,000 reads per cell, the libraries were sequenced using an Illumina NovaSeq 6000. Cell Ranger (v6.1.2) was used to align the raw data to the human GRCh38 reference genome in order to produce expression matrices. Seurat (v4.3.0) was used for downstream analysis in R (v4.2.0): doublets and low-quality cells (<200 or >4000 genes, mitochondrial content >20%) were eliminated, SCTransform was used for normalisation, PCA was used for dimensionality reduction, and the top 30 principal components were used for UMAP visualisation. SingleR and recognised markers were used to annotate the cells. FindMarkers was used to identify genes that were differentially expressed (Wilcoxon rank-sum test, $|\log_2FC| > 0.25$, adjusted $P < 0.05$). ClusterProfiler was used for pathway enrichment analysis. BraCeR was used to reconstruct BCR clonotypes, which were then paired with scRepertoire to determine class-switch distribution and clonal proliferation.

After sorting CD19⁺ B cells from TLS areas of two NSCLC cases, the nuclei were transposed using the Chromium Single Cell ATAC v1.1 kit after being lysed with 0.1% NP-40. Cell Ranger ATAC (v2.0) was used to sequence and analyse the libraries, aligning them to GRCh38. Signac (v1.7.0) was used for downstream analysis. Cells having nucleosome banding patterns and TSS enrichment >4 were filtered before being LSI-normalized for UMAP clustering. GC B cell populations were identified by label transfer using gene activity matrices and scRNA-seq data. Cicero was used to visualise the immunoglobulin heavy chain loci and areas surrounding the AID gene, predict cis-coaccessibility networks, and find differential accessible regions (DARs).

We employed the CUT&Tag procedure that Kaya-Okur et al. published. To put it briefly, about 1×10^5 CD19⁺GL7⁺ GC-B cells were separated from microdissected NSCLC TLS areas, attached to Concanavalin A magnetic beads, permeabilized with 0.05% digitonin, and then incubated with either control IgG or anti-H3K18 lactylation antibody (PTM Bio, 1:50) for an entire night at 4°C. Following washing, they were incubated with the protein A-Tn5 fusion protein (EpiCypher) for an hour before the transposition reaction was triggered by the addition of Mg^{2+} . After DNA extraction and PCR

amplification (18–22 cycles), 1.2× AMPure bead purification was performed. The Illumina platform (PE150, >10M reads/sample) was used to sequence libraries. Trim Galore was used to trim the raw data, Bowtie2 was used to align it to hg38, and MACS2 was used to name peaks at $q < 0.01$. DeepTools was used to build bigWig coverage tracks. DiffBind (DESeq2, FDR < 0.05) was used to identify differential lactylation sites, and ChIPseeker was used to annotate them to the genome.

After extracting the entire protein from recently sorted B cells, we performed SDS-PAGE and transferred it on a PVDF membrane. Following 5% BSA blocking, we incubated anti-pan-lactyl-lysine antibody (1:1000, PTM Bio), anti-H3K18 lactylation (1:1000), anti-AID (1:500, Cell Signalling #4975), and anti- β -Actin (1:5000, Sigma) for an entire night at 4°C. The corresponding HRP secondary antibody and ECL detection. Using the SimpleChIP Enzymatic Chromatin IP Kit (Cell Signalling), we immunoprecipitated either the control H3 antibody or the anti-H3K18 lactylation antibody for ChIP. The AID promoter region, S_{μ} , and $S_{\gamma 2b}$ areas were measured using qPCR after the DNA was purified.

0.5 mg/kg of tamoxifen was administered intraperitoneally to B-Ldha-KO mice (if the inducible system was being used) or continuously expressed in the B cell lineage using Cd19-Cre. To confirm the metabolic inhibition, B cells were separated from the spleen and draining lymph nodes, Ldha mRNA was assessed using qPCR ($\Delta\Delta C_t$ technique), and intracellular lactate levels were assessed using a lactate assay kit (Sigma). TLS B cells in the MC38-OVLactylation levels, AID expression, and CSR efficiency were compared in tumor-bearing animals.

ELISA was used to quantify OVA-specific IgG1, IgG2b/c, and IgA titers in serum and tumour homogenates. Serum was incubated in repeated dilutions on a 96-well plate coated with OVA (10 μ g/mL). Biotinylated anti-mouse IgG1, IgG2c, and IgA secondary antibodies (Southern Biotech) were used for detection, followed by TMB substrate and streptavidin-HRP. Relative affinity was measured by ELISA using NaSCN elution to evaluate affinity maturation. This was represented as the proportion of signal preserved following 2M NaSCN elution. High-throughput sequencing of immunoglobulin heavy chain CDR3 regions in TLS-GC B cells (Illumina MiSeq) was used to analyse the BCR lineage. Clones were assembled using MiXCR, and the Shannon diversity index, clone expansion fractions, and somatic mutation frequency were computed.

Sort CD19⁺ B cells (>95%) magnetically from the tumor-draining lymph nodes or TLS of CD45.2⁺ B-Ldha-KO or control mice, and then inject them intravenously (3×10^6 per mouse) into recipient mice that are CD45.1⁺ wild-type. One day before to receiving anti-PD-1 treatment, the recipient mice received MC38-OVA. Examine TLS development, recipient tumour growth, antibody production, and the location of CD45.2⁺ donor B cells after 14 days.

GraphPad Prism 9.0 or R software were used to analyse all data, which are displayed as mean $\pm$ standard deviation (SD) or median (interquartile range). A two-tailed Student's t-test (for normally

distributed data) or the Mann-Whitney U test were used to compare two groups; one-way ANOVA followed by Tukey's post hoc test was used to compare multiple groups. Two-way repeated measures ANOVA was used to analyse tumour growth curves. The Kaplan-Meier method and the log-rank test were used for survival analysis. Pearson or Spearman correlation coefficients were used to evaluate correlations. Every experiment was conducted independently at least three times, and the figures show the biological replicates. $P < 0.05$, $P < 0.01$, $P < 0.001$, and $P < 0.0001$ were all regarded as statistically significant.

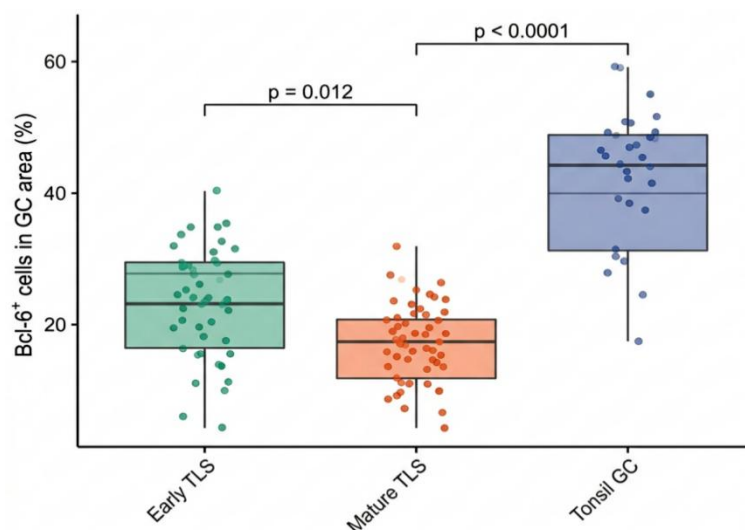
4. Research Results

4.1. Functional exhaustion of B cells in mature TLS is associated with local lactate accumulation

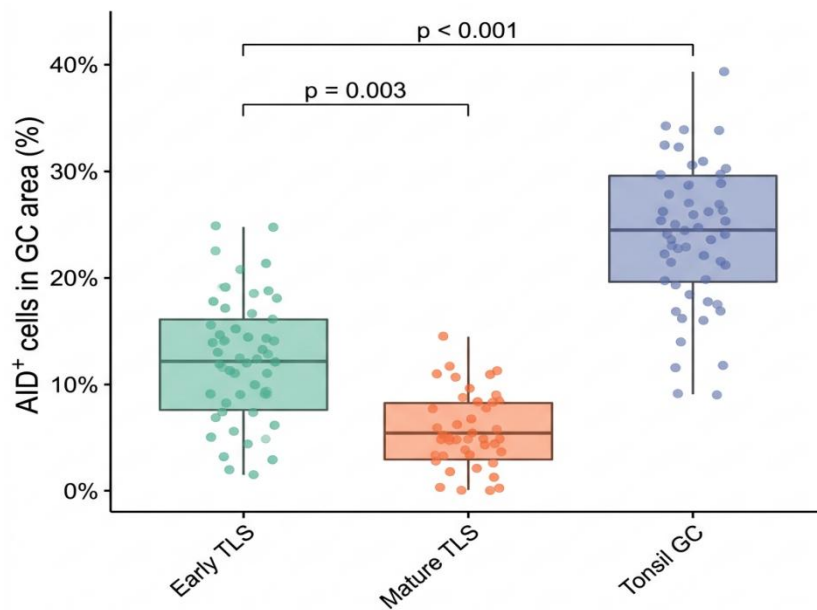
Initially, we performed a systematic multiplex immunofluorescence study on surgical materials from 78 treatment-naïve NSCLC cases in order to investigate the microenvironmental parameters that define B cell functional states within TLS. We classified TLS into early TLS ($n=31$) and mature TLS ($n=47$) based on the presence or absence of $CD21^+$ follicular dendritic cell (FDC) networks and $Bcl-6^+Ki67^+$ germinal centers. The B cell zone ($CD20^+$) in mature TLS had a distinct GC region in the middle and was structurally intact. However, the percentage of $Bcl-6^+$ cells in TLS-GCs was significantly lower ($P=0.003$) than in classic GCs in secondary lymphoid organs, and the frequency of activation-induced cytidine deaminase (AID) positive cells was less than 40% of that in tonsil GCs ($P<0.001$, Figure 1A-C). Furthermore, an obstruction in class switch recombination (CSR) was suggested by the sparse distribution of $IgG2b/c^+$ plasma cells surrounding mature TLS.

Figure 1

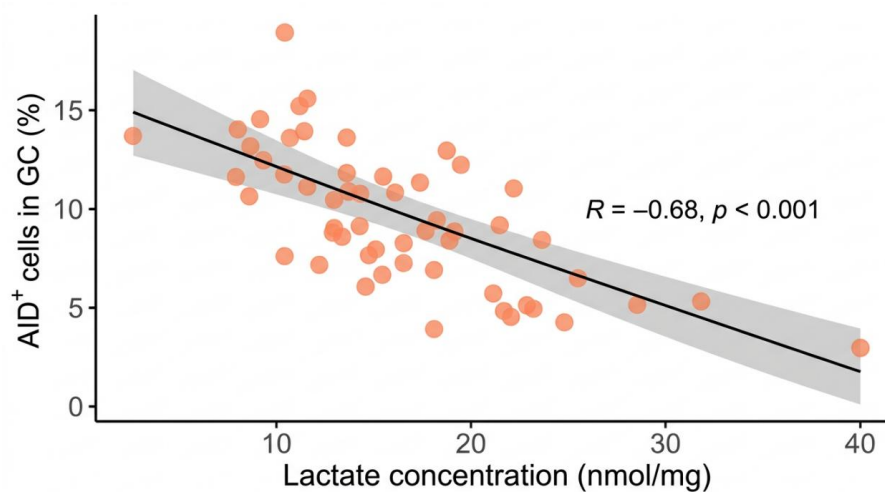
A: Comparison of the proportion of GC-B cells positive for $Bcl-6^+$ across different tissues/structures (box plot + jitter points)



B: Comparison of the proportion of AID-positive cells in different tissues/structures



C: Scatter plot showing the correlation between lactate levels in mature TLS and the proportion of AID-positive cells



We used targeted quantitative metabolomics to evaluate lactate levels in TLS regions in order to determine whether lactate plays a role in the functional fatigue described above. Microdissected mature TLS tissues had a lactate concentration of 28.6 ± 7.3 nmol/mg, which was significantly higher than that of early TLS (15.4 ± 4.1 nmol/mg) and nearby normal lung tissue (6.2 ± 1.8 nmol/mg, all $P < 0.001$, Table 1). This suggests that the immune cells within TLS are exposed to higher lactate levels as TLS matures. Lactate levels in TLS were found to be negatively correlated with both the proportion of Bcl-6⁺ cells ($r = -0.51$, $P = 0.002$) and GC-B cell AID expression ($r = -0.67$, $P < 0.001$), suggesting that lactate accumulation may be a metabolic driver of B cell functional exhaustion.

Table 1

Comparison of lactate levels and B cell function indicators in different tissue areas

Indicator	Normal lung tissue next to the tumor (n=20)	Early TLS (n=31)	Mature TLS (n=47)	P-value
Lactic acid concentration (nmol/mg)	6.2 ± 1.8	15.4 ± 4.1	28.6 ± 7.3	<0.001
AID ⁺ cells/GC area (%)	—	12.3 ± 4.6	5.8 ± 2.9	<0.001
IgG2b/c ⁺ plasma cells per high-power field	0.5 ± 0.3	3.8 ± 1.6	2.1 ± 1.4	0.008
H3K18la immunofluorescence intensity (MFI)	1.0 ± 0.2	3.2 ± 1.1	7.5 ± 2.4	<0.001

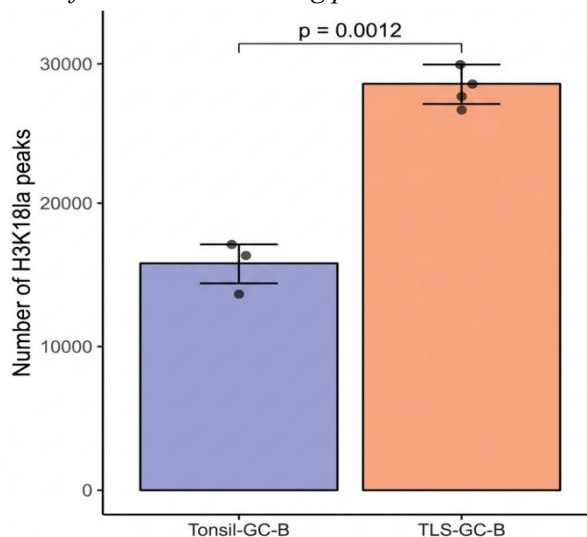
Note. Data are shown as mean ± SD. P values were calculated using one-way ANOVA and Tukey's post hoc test, comparing mature TLS with early TLS.

4.2. TLS-GC-B cells show abnormal genome-wide enrichment of histone H3K18 lactylation

We employed tonsil GC-B cells as a physiological GC control and using CUT&Tag technology to map the genome-wide H3K18 lactylation (H3K18la) in CD19⁺GL7⁺ GC-B cells selected from mature TLS (TLS-GC-B) in order to investigate whether lactate buildup occurs through histone lactylation. Compared to the control group (15,672±1,891, P<0.001), TLS-GC-B cells had a considerably higher number of H3K18la peaks (28,546±3,124) and a higher overall peak signal strength (Fig. 2A). Lactylation may have a direct impact on the start of gene transcription, according to genomic annotation, which revealed that almost 38% of the differential gain peaks in TLS-GC-B cells were found in gene promoter regions. This is significantly greater than the 21% baseline in the control group (P<0.01).

Figure 2

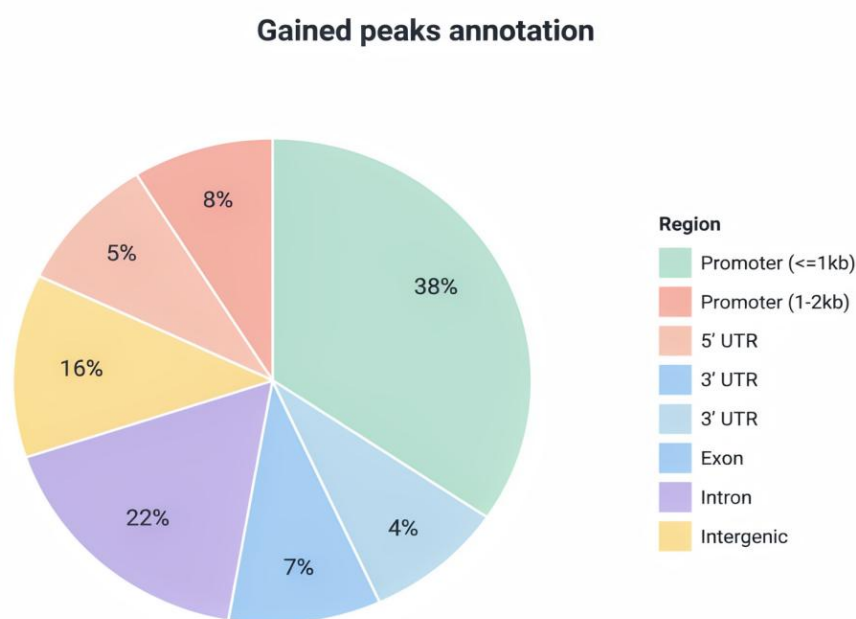
A: Comparison of the number of H3K18la CUT&Tag peaks between TLS-GC-B and Tonsil-GC-B cells



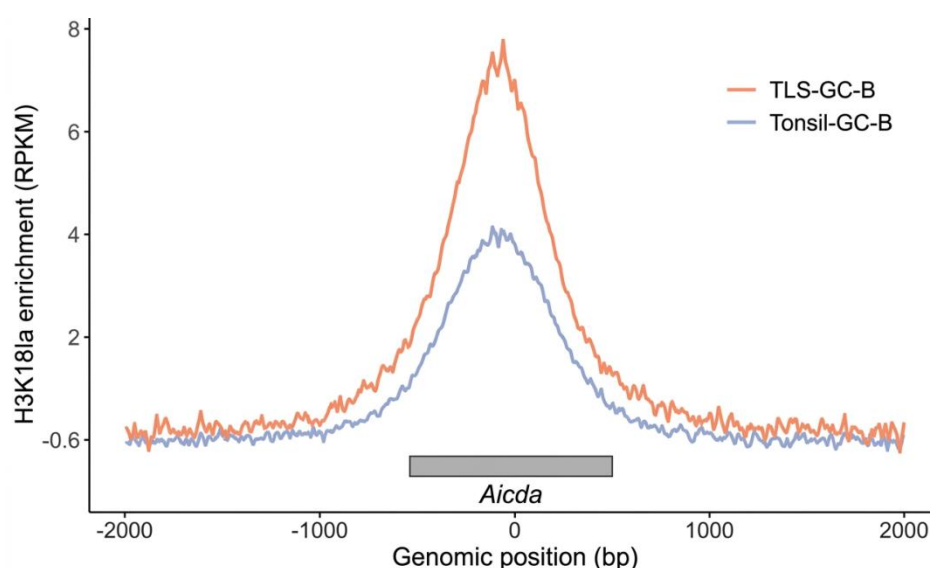
It is noteworthy that there is an aberrant increase in lactylation at the immunoglobulin heavy chain (Igh) constant region locus. The upstream *Sy2b* region of the *Ighg2b/c* gene, the proximal promoter region of *Aicda* (which codes for AID, -0.5 kb to +0.2 kb), and the upstream regulatory area of *Irf4* (Fig. 2B-C, FDR<0.05) were shown to have significantly higher H3K18la signal levels. Track visualisation makes it evident that lactylation changes in these regulatory elements are “island-like” abundant in TLS-GC-B cells, whereas these areas are primarily in a low-modification state in normal GC-B cells. This implies that by focusing on regulatory components of genes linked to antibody maturation, lactate may epigenetically decrease B cell effector capabilities.

Figure 2

B: Pie chart of H3K18la peak genome annotation (TLS-GC-B gained peaks)



C: H3K18la signal tracks at the Aicda locus and Sy2b region

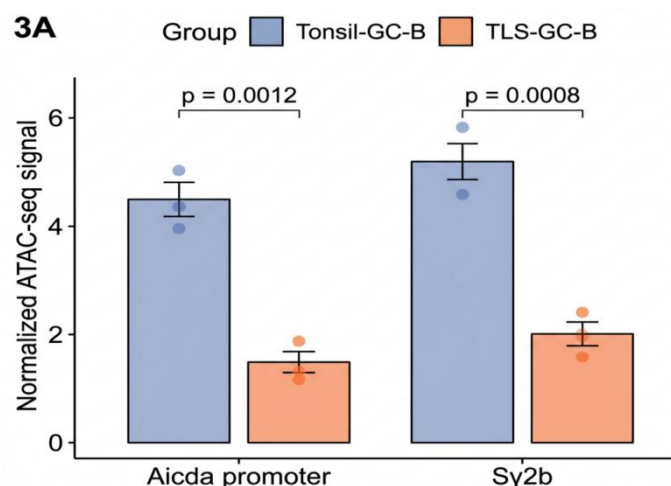


4.3. scATAC-seq reveals chromatin accessibility closure at lactylation target sites

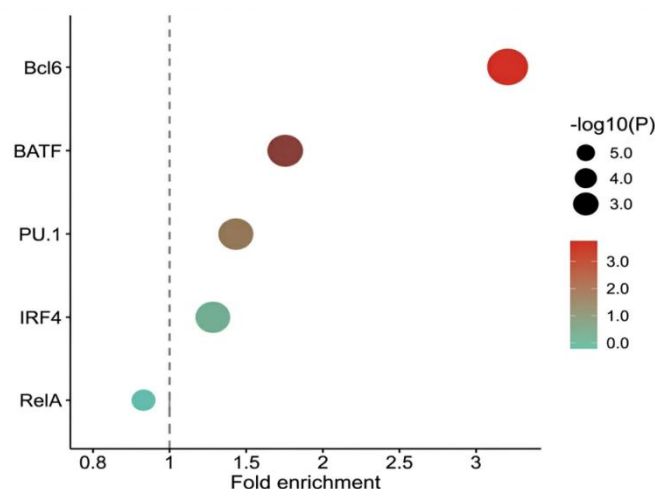
We used parallel scATAC-seq research to comprehend the functional effects of histone lactylation on chromatin structure. The chromatin accessibility of the *Aicda* promoter area and the *Ighg2b/c* S sections was substantially reduced in TLS-GC-B cells compared to normal GC-B cells (FDR<0.01, Figure 3A). We discovered that peaks with elevated H3K18la significantly overlapped with areas of decreased chromatin accessibility by combining CUT&Tag with scATAC-seq data (overlap rate 71.2%, $P<1\times10^{-3}$ by permutation test), indicating a tight relationship between lactylation changes and chromatin “closure.” Further analysis of transcription factor binding motifs showed that motifs for important B cell fate transcription factors Bcl-6, BATF, and PU.1 were significantly enriched ($P<0.001$) in regions with increased lactylation and decreased accessibility, but their predicted DNA binding activity was decreased (Figure 3B). This suggests that histone lactylation may impede transcription factor binding through steric hindrance or co-repressor recruitment.

Figure 3

A: Comparison of chromatin accessibility in the Aicda promoter and Sy2b region



B: Enrichment of transcription factor binding motifs in regions with increased lactylation/reduced accessibility

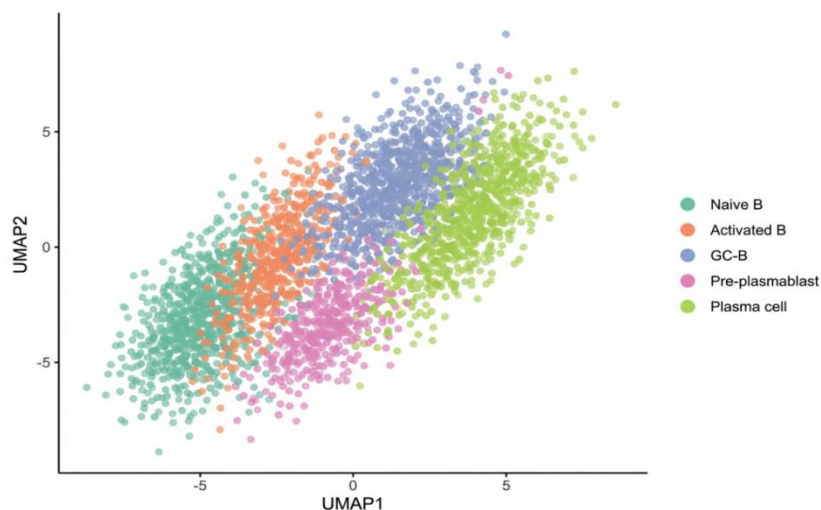


4.4. Single-cell transcriptomics confirms blocked AID expression and CSR defects

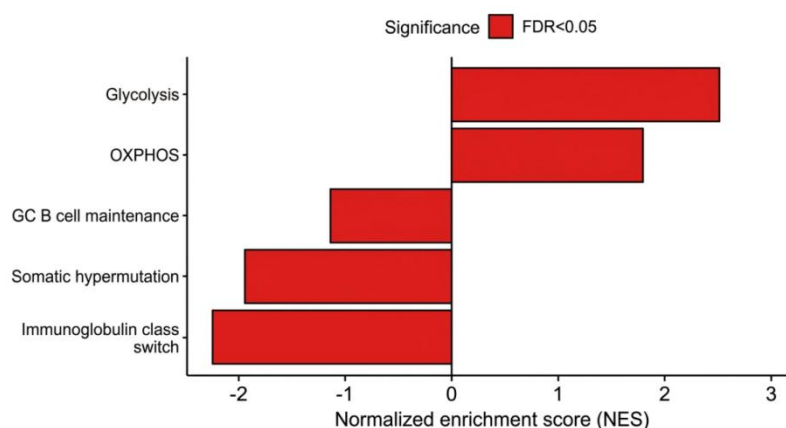
B cells within TLS were separated into five subpopulations by single-cell RNA sequencing analysis: naive B cells, activated B cells, GC-B cells (identified by the expression of Bcl6, Mki67, and Aicda), pre-plasmablasts, and plasma cells (Figure 4A). The “immunoglobulin class switching” and “somatic hypermutation” pathways were considerably decreased in the TLS-GC-B subpopulation when compared to physiological GC, according to differential gene enrichment analysis (NES=-2.3, FDR=0.003, Figure 4B). In TLS-GC-B, Aicda mRNA expression was only 31% of the control level (P<0.001, Table 2). BCR clonal tracking further showed that non-class-switched IgM/D clones accumulated significantly (63.5% vs. 28.3%, Figure 4C), but IgG2b/c clones accounted for just 4.7% in TLS-GC-B, significantly lower than tonsil GC (22.1%). The antibody affinity index (NaSCN elution retention 17.3% vs. 48.6%, P<0.001) and somatic mutation burden (5.2 ± 2.1 mutations/VH area vs. 12.8 ± 3.5) were both significantly decreased.

Figure 4

A: UMAP visualization of B cell subpopulations within TLS



B: GSEA bar chart of differentially expressed genes in GC-B cells (immunoglobulin class switch pathway)



C: Stacked bar chart showing the proportion of different types of immunoglobulins in BCR clones

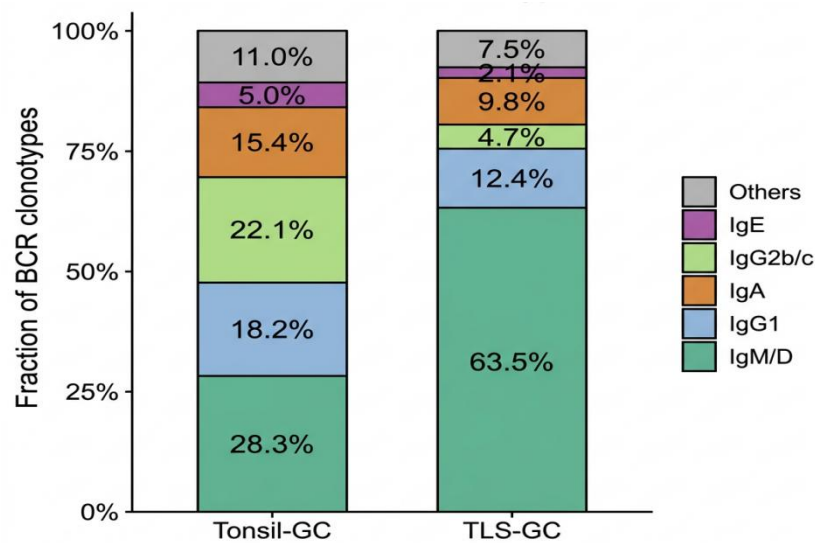


Table 2

Comparison of Key Gene Expression and Functional Indicators Between TLS-GC-B and Physiological GC-B Cells

Indicator	Tonsil GC-B	TLS-GC-B	P-value
Aicda mRNA (log2 normalized counts)	6.8 ± 0.9	3.1 ± 1.1	<0.001
Bcl6 mRNA	5.2 ± 0.7	3.8 ± 0.9	0.004
Irf4 mRNA	4.5 ± 0.8	2.9 ± 0.7	0.002
Proportion of IgG2b/c clone type (%)	22.1 ± 5.4	4.7 ± 2.8	<0.001
Number of somatic mutations/VH region	12.8 ± 3.5	5.2 ± 2.1	<0.001
Affinity retention rate (% , 2M NaSCN)	48.6 ± 8.2	17.3 ± 6.5	<0.001

Note. GC-B cells were sorted from 3 pairs of TLS and 3 independent tonsil samples. Data are shown as mean ± SD, using a two-tailed Student's t-test.

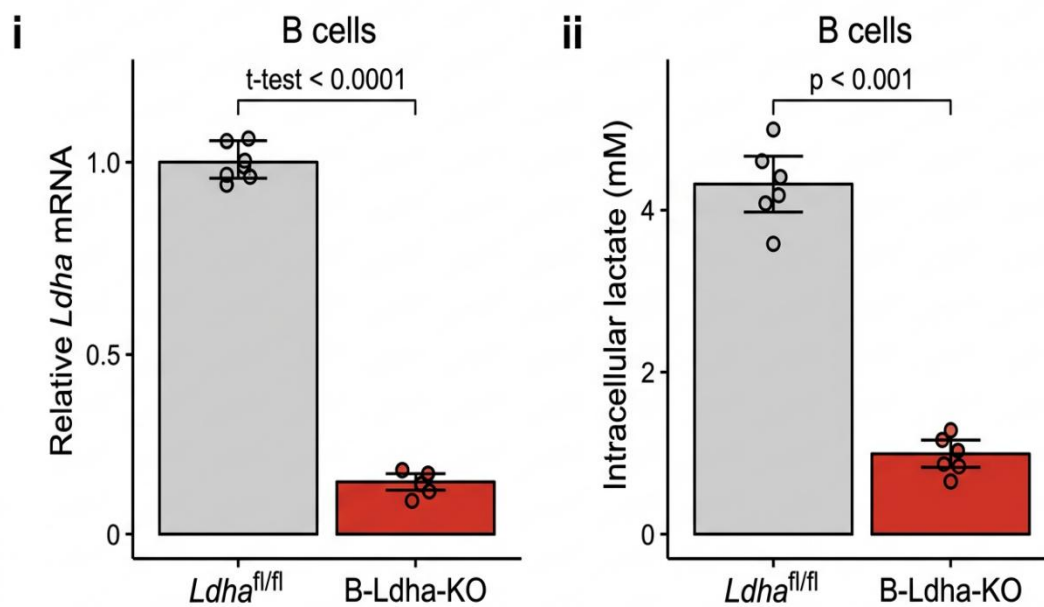
4.5. B cell Ldha deletion eliminates lactate accumulation and restores normal levels of histone lactylation

We produced B cell-specific Ldha knockout mice (B-Ldha-KO) to demonstrate a causal connection. Endogenous lactate metabolism in B cells is the primary cause of histone lactylation, as

demonstrated by validation experiments that showed *Ldha* mRNA levels in the spleen and draining lymph node B cells of B-*Ldha*-KO mice decreased by approximately 87% ($P < 0.001$), intracellular lactate concentration decreased from 4.3 ± 0.8 mM to 0.9 ± 0.3 mM ($P < 0.001$, Fig. 5A-B). H3K18la levels in TLS B cells of B-*Ldha*-KO mice remained similar to physiological GC B cells ($P = 0.78$ vs spleen GC, Fig. 5C), thereby normalising the epigenetic landscape, whereas TLS B cells in control mice under tumor-bearing conditions (MC38-OVA) displayed abnormally high H3K18la consistent with clinical samples.

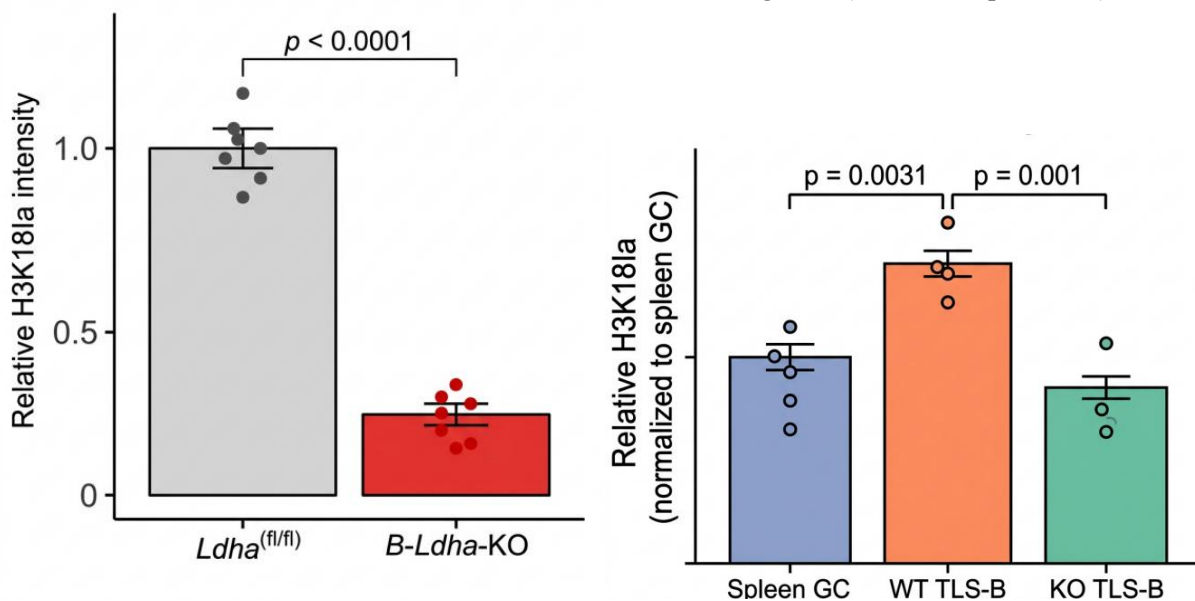
Figure 5

A: *Ldha* mRNA and intracellular lactate levels in B-*Ldha*-KO and control B cells



B: Quantification of overall H3K18 lacylation levels by Western blot

C: Comparison of H3K18la levels in TLS-B cells of tumor-bearing mice (vs normal spleen GC)

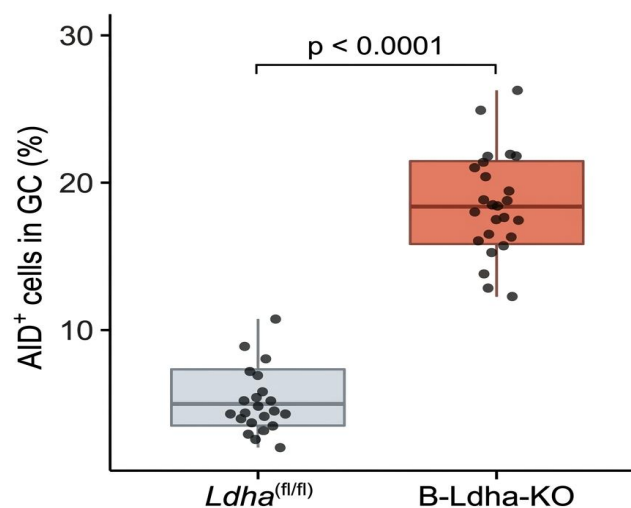


4.6. Ldha deletion restores TLS internal antibody class switching and affinity maturation

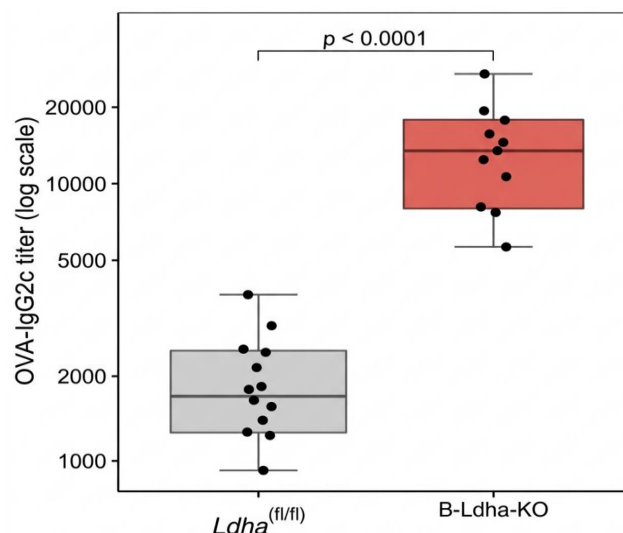
Functionally, the percentage of AID-positive cells in TLS-GC-B cells ($18.5 \pm 4.2\%$) in B-Ldha-KO mice recovered to 3.6 times that of control mice ($5.1 \pm 2.6\%$) ($P < 0.001$, Figure 6A) following tumour formation. $\text{Sy}2\text{b-H3K18la}$ modification was decreased (ChIP-qPCR revealed a 79% decrease, $P < 0.001$) and *Aicda* mRNA expression increased 5.3-fold ($P < 0.001$), whereas chromatin accessibility in the $\text{Sy}2\text{b}$ area was restored (ATAC-qPCR increased 3.1-fold, $P = 0.002$). OVA-specific serum IgG2c titers ($1:12560$ vs. $1:1890$, $P < 0.001$, Table 3) and local tumour IgG2c levels (38.4 ± 9.2 vs. 7.6 ± 3.1 ng/mg tissue, $P < 0.001$) both markedly increased in tandem with the reversal of epigenetic barriers. Relative antibody affinity (retention rate 42.1% vs. 19.7% , $P = 0.002$) and somatic mutation numbers (11.3 ± 3.2 vs. 4.8 ± 2.6 , $P < 0.001$) essentially restored to normal GC levels (Figures 6B–C).

Figure 6

A: Recovery of AID-positive GC-B proportion in TLS



B: Serum OVA-specific IgG2c antibody titer



C: Comparison of somatic mutation count and affinity retention

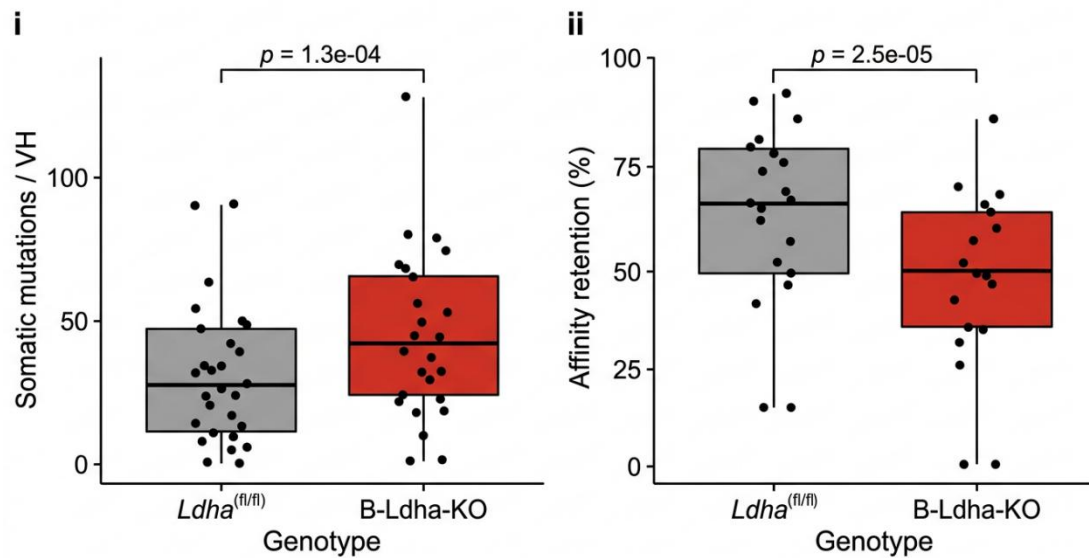


Table 3

Comparison of TLS-B cell function indicators in MC38-OVA tumor-bearing mice

Indicator	Compared with tumor-bearing mice	B-Ldha-KO tumor-bearing mice	Physiological GC reference (spleen)	P value (KO vs Control)
TLS AID ⁺ GC-B (%)	5.1 ± 2.6	18.5 ± 4.2	22.3 ± 3.8	<0.001
Serum OVA-IgG2c titer (¹)	1:1890 ± 860	1:12560 ± 3240	1:14800 ± 2950	<0.001
Tumor OVA-IgG2c (ng/mg)	7.6 ± 3.1	38.4 ± 9.2	—	<0.001
Somatic mutation count/VH	4.8 ± 2.6	11.3 ± 3.2	13.1 ± 2.8	<0.001
Affinity Retention Rate (%)	19.7 ± 7.3	42.1 ± 9.5	51.8 ± 10.2	0.002

Note. Each group had n=8-10 mice. P values were calculated using a two-tailed Student's t-test (KO vs control). ¹Antibody titer is defined as the reciprocal of the highest dilution with OD450 > 0.2.

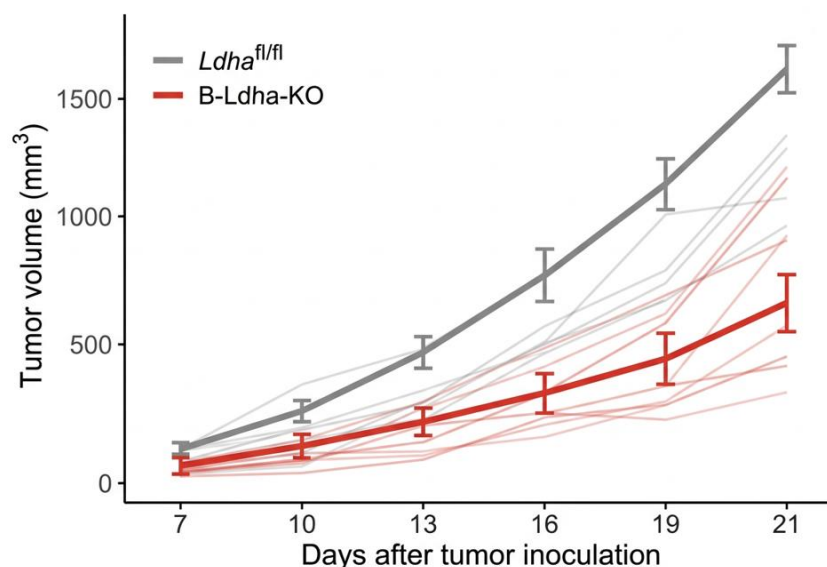
4.7. Metabolic-epigenetic interventions boost anti-tumor immunity and reverse ICB resistance

Lastly, we examined the impact of limiting lactylation recognition or preventing B cell lactate

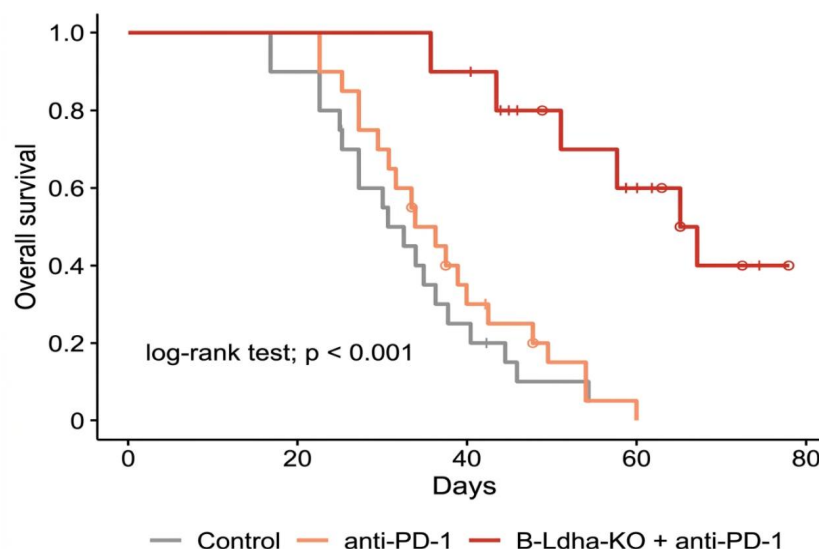
metabolism on tumour growth. After being implanted with MC38-OVA alone, tumours grew more slowly in B-Ldha-KO animals (day 21 volume 456 ± 178 vs. control 1082 ± 256 mm³, $P < 0.001$, Fig. 7A), indicating that TLS B cells with normalised metabolism can naturally provide protective humoral immunity. More significantly, in the PD-1 primary resistant LLC-OVA model, B-Ldha-KO plus anti-PD-1 significantly increased median survival in mice (not reached vs. control 28 days, combo vs. anti-PD-1 alone, $P = 0.002$, Fig. 7B), while PD-1 antibody treatment alone was ineffective (tumour volume difference $P = 0.48$). The presence of donor-derived IgG2c⁺ plasma cells in the tumours was confirmed by adoptive transfer experiments when CD45.2⁺ TLS B cells from B-Ldha-KO mice were transferred into PD-1 resistant CD45.1⁺ recipients. The tumor-suppressive effect could be partially reversed by CD8⁺ T cell depletion (Fig. 7C), suggesting that reconstructed TLS humoral immunity works in conjunction with T cell-mediated killing to exert anti-tumor effects.

Figure 7

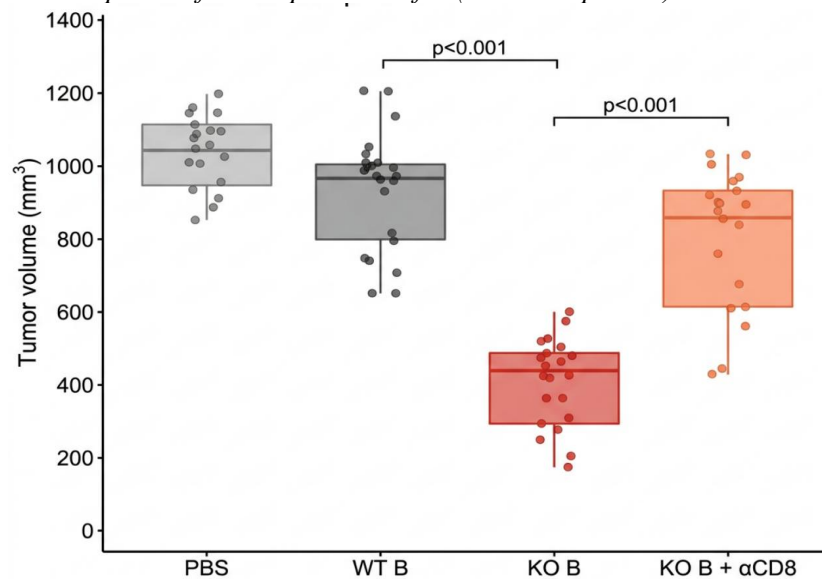
A: Tumor growth curves (Control vs B-Ldha-KO)



B: Survival curve (Kaplan-Meier) — LLC model combined with anti-PD-1

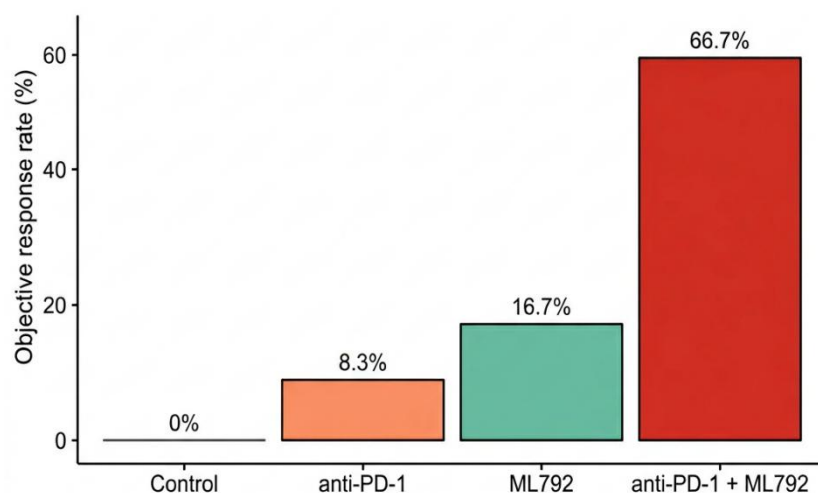


C: Tumor volume in recipients after adoptive transfer (+ CD8 depletion)



We administered the ubiquitin-like modification inhibitor ML792 (an inhibitor of the lactylation recognition complex) to tumor-bearing wild-type mice in order to further confirm this epigenetic target. The B-Ldha-KO phenotype was replicated by ML792 (10 mg/kg, daily intraperitoneal injection), which also reduced H3K18la levels in TLS-B cells, restored AID expression (+3.8 fold, $P<0.001$), raised OVA-specific IgG2c antibody titers, and increased the response rate of anti-PD-1-resistant tumours from 8.3% to 66.7% ($P=0.008$, Fig. 7D). When combined, our findings demonstrate that lactate-driven histone lactylation functions as a targetable metabolic gatekeeper for humoral immunity in TLS B cells, and that inhibiting it can effectively reverse ICB resistance by changing the immunological landscape of the tumour microenvironment.

Figure 7D: Response rate comparison in PD-1 resistant models (ML792 treatment)



5. Discussion

The molecular mechanism underlying humoral immune exhaustion in tumor-infiltrating TLS is

explained from a metabolism-epigenetics interaction perspective by this study, which for the first time demonstrates that lactate-driven histone lactylation functions as a novel metabolic checkpoint for B cell function within tertiary lymphoid structures (TLS). We demonstrate that TLS-GC-B cells absorb abnormally accumulated lactate in the tumour microenvironment, which results in aberrant H3K18 lactylation deposition at important gene loci for antibody class switching and affinity development. This ultimately hinders the generation of protective anti-tumor antibodies by suppressing AID expression, blocking CSR, and causing faulty somatic hypermutation. In addition to providing a new theoretical foundation for combination immunotherapy techniques targeted at restoring TLS function, our discovery closes a long-standing knowledge gap about how the tumour metabolic environment can suppress adaptive humoral immunity in TLS through epigenetic mechanisms.

TLS has long been investigated for its function in adaptive immunological tolerance mechanisms, including T cell exhaustion and regulatory T cell growth, as the center of local adaptive immune responses in tumours. This result is novel because it establishes a direct connection between a fundamental metabolic characteristic of the TME-lactate accumulation-and the functional status of B cells within TLS. Prior research has demonstrated that lactate can polarise TAMs toward the M2 phenotype by stabilising HIF-1 α and directly decreases the cytotoxic capabilities of CD8⁺ T cells and NK cells. But lactate's direct effects on B cells have long been disregarded. The mechanism by which lactate operates as a signalling molecule or epigenetic substrate to regulate B cell effector activities is entirely unclear, however a few reports have suggested that acidic pH can decrease B cell proliferation. This study shows for the first time that lactate precisely reprograms the transcriptional program of GC-B cells through metabolically sensitive histone lactylation instead of pH effects or energy consumption. As a result, lactate's function as a "metabolic waste" or "energy substrate" is essentially expanded to include a "epigenetic switch" for B cell humoral immunity. This finding provides a novel paradigm to understand why TLS struggles to sustain protective roles in a metabolically dysregulated TME and is in accordance with the recent trend in immunometabolism research, which has been spreading beyond T cells and macrophages to B cell lineages.

Research in this field has mostly concentrated on the regulation of tumour cell proliferation and the repair of macrophage inflammation since Zhang et al. identified histone lactylation in 2019. Up until now, the alteration landscape and activities of lymphocytes—particularly B cells undergoing the GC reaction—were entirely unknown. In this work, we mapped the genome-wide H3K18 lactylation landscape in TLS-GC-B cells for the first time by combining CUT&Tag and scATAC-seq analysis. We found important target locations with aberrant lactylation deposition at antibody maturation loci (Ighg2b/c S region, Aicda promoter). It's interesting to note that H3K18la increases significantly overlapped with decreased chromatin accessibility. These regions were enriched with binding motifs for transcription factors that determine the fate of GC-B cells, such as Bcl-6 and BATF, but their

projected binding activity was lowered. This implies that histone lactylation may recruit transcriptional co-repressors with certain “reader” domains or directly prevent transcription factor binding through spatial obstruction, creating a hitherto unidentified “brake” mechanism on antibody gene production. This discovery supports the findings of Moreno-Yruela et al., who demonstrated that HDAC1-3 has histone delactylase activity. In the TLS microenvironment, excessive lactate may overwhelm delactylase activity, resulting in a “write-erase” imbalance. Under normal GC conditions, delactylases may maintain the epigenetic “open” state of loci like AID to ensure antibody diversity. It is imperative that future research determine the kinds of acyltransferases that mediate B cell lactylation “writing” and whether “reader” proteins exist that can identify lactylation at particular loci.

The majority of patients with solid tumours have either primary or acquired resistance, which is one of the fundamental obstacles to current ICB therapy. Our clinical data indicates that even mature TLS frequently include B cell zones in a functionally “silent” condition, despite the fact that several seminal studies have demonstrated that TLS is an independent positive predictor of ICB response. Because the local metabolic environment suppresses the main B cell effector arm, this may help explain why some TLS-positive tumours still do not respond to ICB. Lifting the suppression of histone lactylation can successfully restore class switching and affinity maturation in TLS-GC-B cells, converting “cold” tumours that are primarily resistant to anti-PD-1 into “hot” tumours and achieving synergistic effects with ICB. This was confirmed in this study using the B-Ldha-KO model and pharmacological intervention (ML792). The therapeutic conclusion here is that assessing TLS as “present or absent” may not be sufficient; their “functional state” is as important. Reactivating “silent TLS” may be accomplished by focusing on lactate metabolism (e.g., LDH inhibitors) or lactylation recognition complexes. Interestingly, adoptive transfer investigations revealed that normalised TLS-B cells’ anti-tumor antibodies partially depend on CD8⁺ T cells to suppress tumours, indicating a synergistic amplification loop between T cell immunity and antibody-dependent cell-mediated cytotoxicity (ADCC). This emphasises even more the importance of combination treatments meant to restore B cell function in TLS.

There are a number of limitations to this study. Initially, we concentrated primarily on H3K18 lactylation; however, lactylation can occur on other histone lysine residues (e.g: H3K9, H4K12, etc.), and changes at various places may have distinct or even conflicting consequences. Future studies should focus on mapping the lactylation code in TLS-B cells more thoroughly. Second, it is still unknown which particular transporters deliver lactate into B cells and whether intracellular lactate compartmentalisation influences the specificity of the modification, despite our confirmation that lactate is transformed by LDHA into lactylation substrates to drive the modification. Third, the main findings must be verified in spontaneous tumour models or genetically modified mice because our mouse model employed subcutaneous tumour transplants and the TLS generation process and TME

features differ from human spontaneous tumours. Fourth, prospective research is needed to determine how TLS-B cell lactylation changes dynamically following chemo/radiotherapy or ICB exposure, given the clinical sample size was modest and only comprised untreated patients. Lastly, ML792's pharmacological validation was merely a proof-of-concept for the target; a thorough evaluation of its selectivity, pharmacokinetics, and possible toxicity is required, and the development of B cell-targeted delivery vehicles may further increase the therapeutic window.

6. Conclusion

The “lactate-histone lactylation” axis has never before been identified as a crucial metabolic checkpoint for B cell humoral immunity in tertiary lymphoid structures (TLSs). We demonstrate that germinal center B cells absorb abnormally accumulated lactate in the tumour microenvironment, which results in aberrant H3K18 lactylation deposition at crucial genetic loci for immunoglobulin class switch recombination and somatic hypermutation, such as the Aicda promoter and Sγ2b region. Protective humoral immunity within TLSs is ultimately functionally “silenced” as a result of this epigenetic remodelling, which inhibits the production of activation-induced cytidine deaminase (AID), preventing IgG2b/c class switching and affinity maturation. These epigenetic barriers can be effectively removed by pharmacologically blocking the lactylation recognition complex or conditionally knocking out lactate dehydrogenase A in B cells to stop endogenous lactate production. This will restore germinal center responses and high-affinity antibody production in TLSs and work in concert with immune checkpoint blockade therapy to reverse primary resistance. These results provide specific targets for TLS-focused combination immunotherapy and reveal a novel pathogenic mechanism by which the tumour metabolic microenvironment “hijacks” adaptive humoral immunity via epigenetic pathways. They also broaden the functional scope of histone lactylation from macrophages and tumour cells to the B cell lineage.

The following areas should be the focus of future research: mapping a thorough atlas of multi-site histone lactylation in TLS-B cells, understanding the intricacy of the “lactylation code” and the interactions between sites; methodically identifying the entire molecular machinery that mediates the writing, erasing, and reading of B cell lactylation, elucidating the targeted regulatory mechanisms of acyltransferases like p300/CBP and specific delactylases at antibody maturation loci; validating lactylation modification as a predictive biomarker for ICB efficacy using spontaneous tumour models and clinical cohorts; and assessing the safety and efficacy of combining targeted lactylation pathways with immunotherapy; creating integrated methods to study the spatiotemporal dynamics of metabolism-epigenetic networks within TLS in situ using single-cell spatial metabolomics and epigenomics. In addition to providing crucial scientific evidence for the development of next-generation immunotherapy techniques based on TLS function restoration, advancing these

investigations will expand our understanding of the metabolic regulation of the local immune milieu.

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Author contributions

Conceptualization, Gang Liu; methodology, Alena Mikalaeuna Vasiukevich; validation, Jia Wang; formal analysis, Gang Liu and Jia Wang; investigation, Alena Mikalaeuna Vasiukevich and Jia Wang; resources, Gang Liu; data curation, Alena Mikalaeuna Vasiukevich; writing—original draft preparation, Jia Wang; writing—review and editing, Gang Liu and Alena Mikalaeuna Vasiukevich; visualization, Jia Wang; supervision, Gang Liu; project administration, Gang Liu. All authors have read and agreed to the published version of the manuscript.

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Data availability

Not applicable.

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Conflict of interest

The authors declare that there is no conflict of interest.

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

All authors have given their consent.

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References

- [1] Angelin, A., Gil-de-Gómez, L., Dahiya, S., et al. (2017). Foxp3 reprograms T cell metabolism to function in low-glucose, high-lactate environments. *Cell Metab*, 25(6), 1282-1293.e7. <https://doi.org/10.1016/j.cmet.2016.12.018>
- [2] Bolland, D. J., Koohy, H., Wood, A. L., Matheson, L. S., Krueger, F., Stubbington, M. J., Baizan-Edge, A., Chovanec, P., Stubbs, B. A., Tabbada, K., Andrews, S. R., Spivakov, M., & Corcoran, A. E. (2016). Two mutually exclusive local chromatin states drive efficient V(D)J recombination. *Cell Rep*, 15(11), 2475-2487. <https://doi.org/10.1016/j.celrep.2016.05.020>
- [3] Brand, A., Singer, K., Koehl, G. E., et al. (2016). LDHA-associated lactic acid production blunts tumor immunosurveillance by T and NK cells. *Cell Metab*, 24(5), 657-671. <https://doi.org/10.1016/j.cmet.2016.08.011>
- [4] Buenrostro, J. D., Wu, B., Litzenburger, U. M., et al. (2015). Single-cell chromatin accessibility reveals principles of regulatory variation. *Nature*, 523(7561), 486-490. <https://doi.org/10.1038/nature14590>
- [5] Cabrita, R., Lauss, M., Sanna, A., et al. (2020). Tertiary lymphoid structures improve immunotherapy and survival in melanoma. *Nature*, 577(7791), 561-565. <https://doi.org/10.1038/s41586-019-1914-8>
- [6] Chen, Z., & Wang, J. H. (2019). Signaling control of antibody isotype switching. *Adv Immunol*, 141, 105-164. <https://doi.org/10.1016/bs.ai.2019.01.001>
- [7] Colegio, O. R., Chu, N. Q., Szabo, A. L., et al. (2014). Functional polarization of tumour-associated macrophages by tumour-derived lactic acid. *Nature*, 513(7519), 559-563. <https://doi.org/10.1038/nature13490>
- [8] Dai, X., Lv, X., Thompson, E. W., & Ostrikov, K. K. (2022). Histone lactylation: Epigenetic mark of glycolytic switch. *Trends Genet*, 38(2), 124-127. <https://doi.org/10.1016/j.tig.2021.09.009>
- [9] Dieu-Nosjean, M. C., Antoine, M., Danel, C., et al. (2008). Long-term survival for patients with non-small-cell lung cancer with intratumoral lymphoid structures. *J Clin Oncol*, 26(27), 4410-4417. <https://doi.org/10.1200/JCO.2007.15.0284>



- [10] Ersching, J., Efeyan, A., Mesin, L., Jacobsen, J. T., Pasqual, G., Grabiner, B. C., Dominguez-Sola, D., Sabatini, D. M., & Victora, G. D. (2017). Germinal center selection and affinity maturation require dynamic regulation of mTORC1 kinase. *Immunity*, 46(6), 1045-1058.e6. <https://doi.org/10.1016/j.immuni.2017.06.005>
- [11] Fridman, W. H., Meylan, M., Petitprez, F., Sun, C. M., Italiano, A., & Sautès-Fridman, C. (2022). B cells and tertiary lymphoid structures as determinants of tumour immune contexture and clinical outcome. *Nat Rev Clin Oncol*, 19(7), 441-457. <https://doi.org/10.1038/s41571-022-00619-z>
- [12] Germain, C., Gnjatich, S., Tamzalit, F., et al. (2014). Presence of B cells in tertiary lymphoid structures is associated with a protective immunity in patients with lung cancer. *Am J Respir Crit Care Med*, 189(7), 832-844. <https://doi.org/10.1164/rccm.201309-1611OC>
- [13] Helmink, B. A., Reddy, S. M., Gao, J., et al. (2020). B cells and tertiary lymphoid structures promote immunotherapy response. *Nature*, 577(7791), 549-555. <https://doi.org/10.1038/s41586-019-1922-8>
- [14] Jellusova, J., & Rickert, R. C. (2016). The PI3K pathway in B cell metabolism. *Crit Rev Biochem Mol Biol*, 51(5), 359-378. <https://doi.org/10.1080/10409238.2016.1215288>
- [15] Kaya-Okur, H. S., Wu, S. J., Codomo, C. A., et al. (2019). CUT & Tag for efficient epigenomic profiling of small samples and single cells. *Nat Commun*, 10(1), 1930. <https://doi.org/10.1038/s41467-019-09982-5>
- [16] Laumont, C. M., Banville, A. C., Gilardi, M., Hollern, D. P., & Nelson, B. H. (2022). Tumour-infiltrating B cells: Immunological mechanisms, clinical impact and therapeutic opportunities. *Nat Rev Cancer*, 22(7), 414-430. <https://doi.org/10.1038/s41568-022-00466-1>
- [17] Moreno-Yruela, C., Bæk, M., Monda, F., et al. (2022). Class I histone deacetylases (HDAC1–3) are histone lysine deacetylases. *Sci Adv*, 8(3), eabi6696. <https://doi.org/10.1126/sciadv.abi6696>
- [18] Pavri, R., Gazumyan, A., Jankovic, M., et al. (2010). Activation-induced cytidine deaminase targets DNA at sites of RNA polymerase II stalling by interaction with Spt5. *Cell*, 143(1), 122-133. <https://doi.org/10.1016/j.cell.2010.09.017>



- [19] Petitprez, F., de Reyniès, A., Keung, E. Z., et al. (2020). B cells are associated with survival and immunotherapy response in sarcoma. *Nature*, 577(7791), 556-560. <https://doi.org/10.1038/s41586-019-1906-8>
- [20] Stubbington, M. J. T., Rozenblatt-Rosen, O., Regev, A., & Teichmann, S. A. (2017). Single-cell transcriptomics to explore the immune system in health and disease. *Science*, 358(6359), 58-63. <https://doi.org/10.1126/science.aan6828>
- [21] Wang, Z. H., Peng, W. B., Zhang, P., et al. (2021). Lactate in the tumour microenvironment: From immune modulation to therapy. *EBioMedicine*, 73, 103627. <https://doi.org/10.1016/j.ebiom.2021.103627>
- [22] Warburg, O. (1956). On the origin of cancer cells. *Science*, 123(3191), 309-314. <https://doi.org/10.1126/science.123.3191.309>
- [23] Yu, J., Chai, P., Xie, M., et al. (2021). Histone lactylation drives oncogenesis by facilitating m⁶A reader protein YTHDF2 expression in ocular melanoma. *Genome Biol*, 22(1), 85. <https://doi.org/10.1186/s13059-021-02308-z>
- [24] Zhang, D., Tang, Z., Huang, H., et al. (2019). Metabolic regulation of gene expression by histone lactylation. *Nature*, 574(7779), 575-580. <https://doi.org/10.1038/s41586-019-1678-1>