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Integrative Bioinformatics Reveals the Lactate Dehydrogenase B (LDHB) Significance in Colon Adenocarcinoma

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Background: Lactate dehydrogenase B (LDHB), a typical oxidoreductase for converting lactate to pyruvate in the glycolysis process, takes a complex function in the progression of cancer cells. Even so, the profile of LDHB relevance in colon adenocarcinoma (COAD) remains ambiguous. Hence this study analyzed the expression and co-expression profile of LDHB, and its immune correlation in COAD.

Materials and method: The mRNA expression and co-expression of LDHB in COAD were retrieved from UALCAN. The immune infiltration levels of LDHB from B cells, $CD4^+$ T cells, $CD8^+$ T cells, macrophages, neutrophils, and dendritic cells in COAD were assessed using the TIMER database. For assessing gene ontology and the KEGG pathway, DAVID v6.8 was utilized. The protein-protein interaction of LDHB-correlated genes was analyzed using STRINGDB and Cytoscape.

Results: Significantly high expression of LDHB in COAD was spotted in several sample types and associated with a poor overall survival rate. Further, LDHB corresponded to the level of $CD4^+$, macrophages, and myeloid-derived suppressor cell (MDSC) immune infiltrating cells. The co-expression of LDHB was associated with several essential genes for cell cycle progression.

Conclusion: The findings of this study indicate an upcoming involvement of LDHB in COAD tumorigenesis and prognosis. Additionally, this study highlights the immune correlation of LDHB in COAD as preliminary data in developing diagnosis and treatment with a novel immune checkpoint in COAD.

Keywords: *lactate dehydrogenase, colon adenocarcinoma, expression, survival, immune*

Introduction

A class of colorectal cancer, colon adenocarcinoma (COAD), still became the top 3 cancer-related deaths worldwide.[1] Until now, common treatments of COAD, such as single and combined chemotherapy, radiotherapy, and surgery, faced numerous difficulties in combating cancer cells and a high probability of relapse.[1] The complexity of diagnostic, therapy, and molecular signaling pathways challenges us to figure out an exciting marker to enhance the prognosis of COAD survivors.[1,2] COAD is broadly known for its unique molecular pathway, including the aberration of CpG

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island methylator phenotype (CIMP) and the instability of chromosomal and microsatellites.[3] Numerous recent studies have reported that the complex dynamic cellular environment or tumor microenvironment (TME) significantly controls COAD progression.[4] The cellular component-cancer cell interaction reorganizes the tumor microenvironment (TME) to help cancer cells satisfy their high metabolic appetites and overall tumor growth.[4] Understanding the TME could also be needed to identify predictive biomarkers given the complexity of systemic immune and nonimmune factors in cancer immunotherapy.[5] A specific dysregulation of TME in the cancer metabolic process, glycolysis, is termed the Warburg effect.[4] Furthermore, alterations in lactate-metabolism in COAD contribute to the tumor progression and high levels of lactate dehydrogenase were correlated with unfavorable overall survival.[6]

The glycolytic enzyme lactate dehydrogenase (LDH) comprises two distinct subunits, namely LDHA and LDHB, and converts pyruvate and lactate.[4] LDHA and LDHB are paralog genes class and known to possess an identical activity as an oxidoreductase. LDHA tends to convert pyruvate to lactate in an anaerobic environment, whereas LDHB showed a higher preference for converting lactate to pyruvate when oxygen is overflowed.[7] The exploration of LDH's significance in cancer progression has been extensively executed in recent years. LDHB levels exhibit variation across diverse cancer types, including within cancer cell lines derived from identical tumor types.[8] In contrast, LDHA is expressed at a notably high level in nearly all cancer cells; for example, overexpression of LDHA in COAD is associated with undesirable survival outcomes and immune infiltration.[8] These fluctuating expressions of LDHB may accommodate the probability of promoting LDHB as a target to treat specific cancer types. Increased lactate metabolism in COAD was associated with the development of an immunosuppressive tumor microenvironment, which led to immune evasion and a poor prognosis.[9] Lactate dehydrogenase, especially LDHB, found to be involved in controlling lysosomal activity and autophagy enabling oxidative phenotype cancer cells to use lactate preferentially over glucose, leading to cell proliferation in both types of cells.[4] The association between LDHB and metabolic dysregulation in COAD, including reactive oxygen species and high basal activity in cancer cells[4], is challenging to study, considering LDHB generally converts nicotinamide adenine dinucleotide $(NAD)^+$ to NADH in a high abundance of oxygen. In most studies, LDHB showed closely related to the malignant progression but still provides a vague mechanistic understanding of how LDHB is associated with cancer cell death, apoptosis, autophagy, and overall TME reprogramming.[10] However, the expression and co-expression profile of LDHB, especially in COAD, and its involvement in the immune response have yet to be fully documented.

In this study, the expression, co-expression, and immune correlation of LDHB in COAD through an integrative bioinformatic was analyzed. The objective of the study was to establish the significance of LDHB in COAD and present preliminary data as supporting evidence for its potential in clinical utilization.

Materials and methods

mRNA Expression Level of LDHB and Its Survival Estimation

The TIMER 2.0 tools (*http://timer.cistrome.org/*)[11] were implemented to examine the LDHB mRNA expression levels in various cancer types across all The Cancer Genome Atlas (TCGA) tumor data using the default setting. The Wilcoxon test to assess the statistical difference between the tumor and normal of each cancer type was applied. Illustration of gene expression level distribution was presented using box plots.

The survival outcome estimation of LDHB expression level in COAD was observed using the TIMER database according to the TCGA dataset[11], using applied setting of Coxph Model, Surv(OS, EVENT) ~ Gene + Stage + Purity + Race + Gender + Age. The COAD samples were classified into high and low expression in Transcripts per Million (TPM). The probability of survival (p<0.05) was calculated using the Kaplan–Meier method.

LDHB mRNA Expression

The UALCAN database (*https://ualcan.path.uab.edu/*) examined the LDHB mRNA expression levels in several colon cancer sample types (primary tumor, adenocarcinoma, mucinous adenocarcinoma, cancer in stage 1 until 4, metastatic, and TP53-mutant cancer).[12] The setting: |Log2FC| cutoff: 2 and p<0.05 was employed, and the Human Protein Atlas datasets were used to display the panel of LDHB expression in immunohistochemistry.

Correlation between LDHB and Immune Infiltration Level

TIMER[11] was used to compare LDHB and immune infiltration levels from B cells, $CD4^+$, T cells, $CD8^+$ T cells,

macrophages, neutrophils, and dendritic cells in COAD. A scatter plot was used to present the relationship of LDHB and infiltrates immune cells in COAD based on purity-adjusted Spearman's rho.

Gene Ontology of LDHB Correlated Genes

We utilized the UALCAN dataset to explore the list of genes that exhibit positive and negative correlations with LDHB in COAD.[12] The correlated genes were statistically analyzed using Pearson-CC value, and the gene with very low expression (Median TPM<0.5) was taken off the list and filtered Person-CC value >|0.3|.

The gene ontology of LDHB-associated genes, comprising biological process, molecular function, cellular component, and KEGG pathway analysis, were screened using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) v6.8 with a $p<0.05$ (*https://david.ncifcrf.gov/content.jsp?file=release.html*).

Protein-protein Interaction and Top 10 Genes

The STRINGDB platform, accessible at *https://string-db.org/cgi/network*, were used to evaluate the chain connection between correlated genes. The cutoff criteria used in this analysis was a confidence score of > 0.4 and the maximum number of interactors = 0. We used the Cytoscape plugin to elucidate the biological significance among network genes by applying degree cutoff = 2, node score cutoff = 0.2, k-core = 2, and maximum depth = 100. The top 10 genes were obtained from filtering using a confidence score > 0.4 and the maximum number of interactors > 5 in CytoHubba plugins.

Results

mRNA Level of LDHB Expression in Pan-cancer TCGA Samples

The differential expression of LDHB in pan-cancer was explored by the TIMER dataset by comparing the tumor *vs.* normal group and calculating its significance by statistical analysis (Wilcoxon test). The LDHB expression was displayed in Transcripts per million [log2 TPM] values. LDHB mRNA expression was significantly up-regulated ($p<0.001$) in COAD, lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), head and neck cancer (HNSC), and cholangiocarcinoma (CHOL). In invasive breast carcinoma (BRCA), kidney chromophobe (KICH), kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KRIP), and prostate adenocarcinoma (PRAD), LDHB mRNA expression was considerably down-regulated ($p<0.001$) (Figure 1A). It was found that in COAD, the LDHB expression in tumor (n=457) *vs.* normal (n=41) was significantly overexpressed with the $p<0.001$. In this study, we continue our deepening focus on COAD.

Since the expression of LDHB was increased significantly in COAD patients, the survival estimation of LDHB expression was obtained from the TIMER database according to the TCGA dataset. COAD accompanied by elevated LDHB expression showed substantially poorer cumulative survival than COAD with low LDHB expression (Figure 1B), which had more remarkable cumulative survival ($p<0.05$). The survival estimation of LDHB expression displayed an exciting challenge in exploring LDHB in COAD.

LDHB mRNA Expression in Various Sample Types of COAD

For further comprehension, the expression level of COAD in several sample types, including histological subtypes, pathological stages, p53 mutation status, and nodal metastasis formation, using different online tools, the UALCAN database, were analyzed. The expression of LDHB (TPM) in the primary tumor (n=286) was significantly ($p<0.001$) higher than normal (n=41) (Figure 2A), confirming the results from the TIMER dataset above. According to the histological subtypes sample (Figure 2B), LDHB was found to be significantly higher ($p<0.001$) in both adenocarcinoma (n=243) and mucinous adenocarcinoma (n=37) compared to normal (n=41). In detail, LDHB was found to be significant ($p<0.001$) in all tumor stages, TP53-mutant, TP53-non-mutant, and all metastasis nodal (Figure 2C-2E). The immunohistochemistry of LDHB in normal and tumor tissue was also presented (Figure 2F), and significant differences in appearance were spotted. The result of this study validated that LDHB was significantly upregulated or overexpressed in all COAD sample cases.

Immune Cell Infiltration of LDHB Expression in COAD

The relationship between LDHB and immunocyte infiltration to learn more about the function of the immunological environment in the development and prognosis of COAD was also investigated. The expression of LDHB was shown to be independently related to the amount of infiltration of ten distinct immune cells, including neutrophils,

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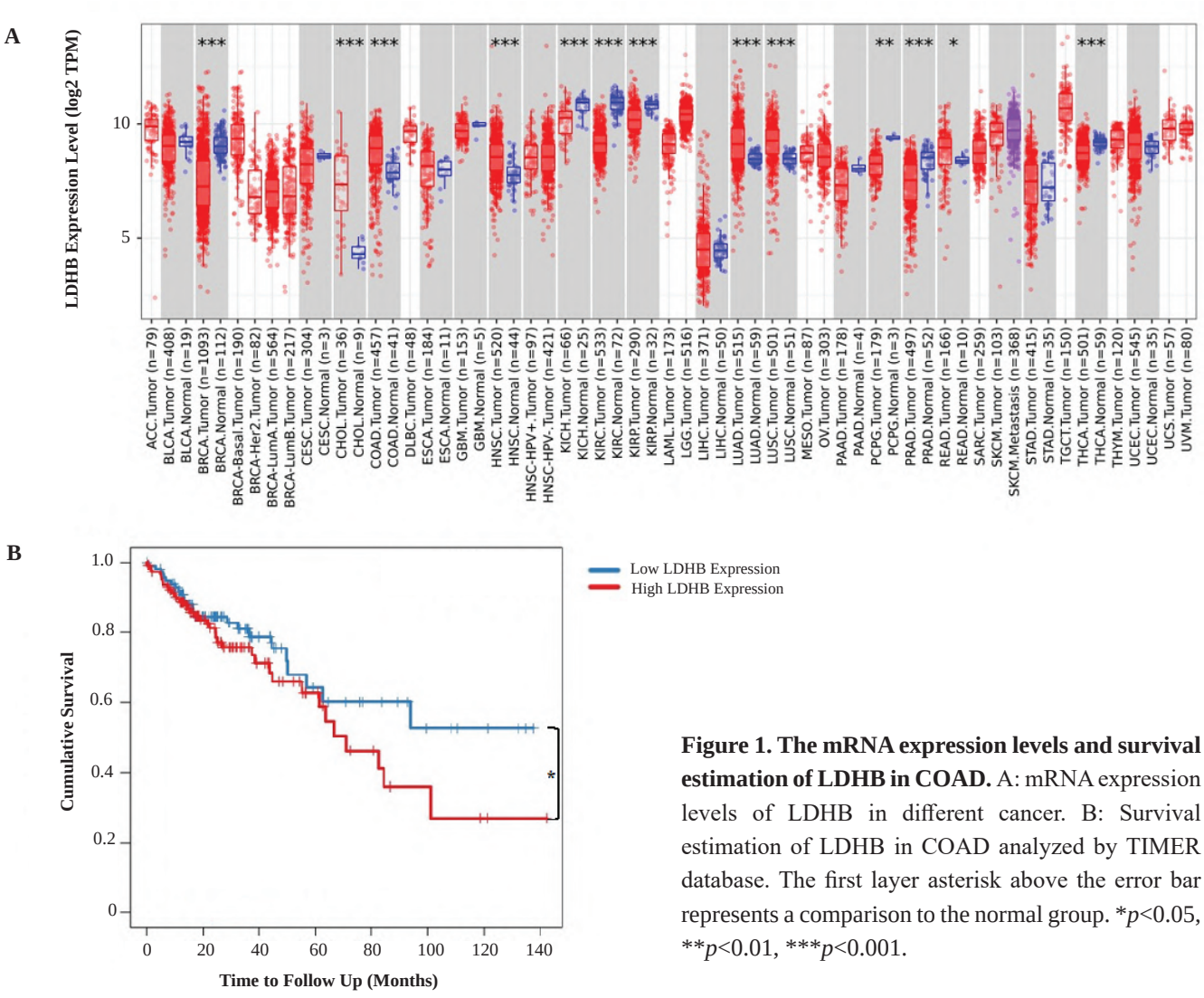

Figure 1. The mRNA expression levels and survival estimation of LDHB in COAD. A: mRNA expression levels of LDHB in different cancer. B: Survival estimation of LDHB in COAD analyzed by TIMER database. The first layer asterisk above the error bar represents a comparison to the normal group. *p<0.05, **p<0.01, ***p<0.001.

macrophages, dendritic cells (DC), CD4$^+$, CD8$^+$, B cells, mast cells, myeloid-derived suppressor cells (MDSC), T cell regulatory (Tregs), and natural killer (NK) cells. Our results indicate LDHB has a regulatory effect on the immunological microenvironment through its impact on the infiltration of various immune cells, including CD4$^+$ T cells, macrophages, MDCS, mast cells, DC, CD8$^+$ T cells, Tregs, and NK cells. A positive correlation was observed between high expression levels of LDHB and increased infiltration of CD4$^+$ cells, macrophages, MDCS, and mast cells. Furthermore, a negative correlation was observed between LDHB and DC, Tregs, and NK cells, indicating that low LDHB expression is associated with high infiltration of these immune cells (Figure 3). Thus, the findings provided additional evidence that LDHB expression was associated with immune cell infiltration in COAD. The TME of LDHB served a crucial role in facilitating immune evasion. LDHB might impact the regulation of immune cell infiltration of varying phenotypes, potentially influencing the onset and progression of COAD.

Co-Expression Network of LDHB in COAD

LDHB was found to be essential and significant in COAD progression. Since the cancer progression is driven by multi-signaling by several genes, we observed the positive and negative genes which correlated to the LDHB signaling pathway. The expression level of positively (n=100) and negatively (n=100) correlated genes was displayed in a heatmap (Supplementary Figure 1). Several genes also found to be overexpressed in COAD compared with normal tissue. For the gene ontology and KEGG pathway, we analyzed a total of 932 genes of positively (n=832) and

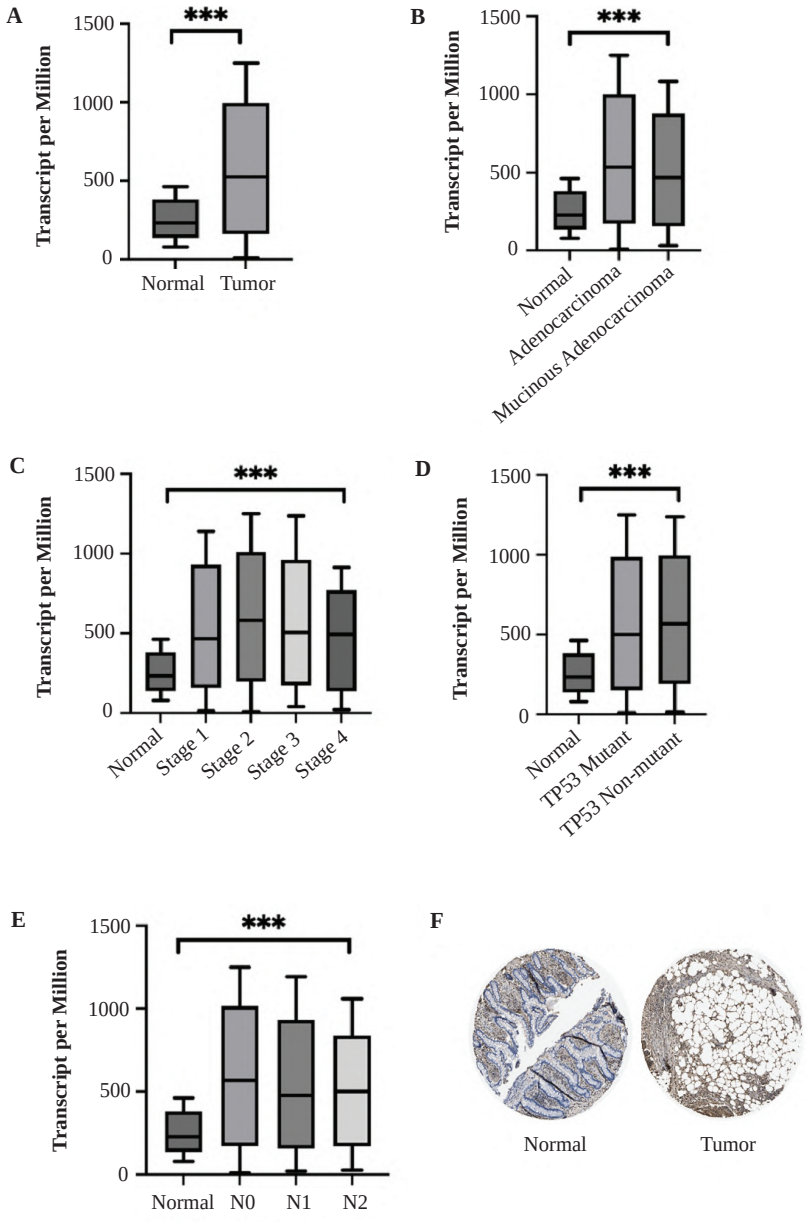

Figure 2. The relationship between LDHB expression levels in several sample types. A: Normal *vs.* primary tumor. B: Histological subtypes. C: Pathological stages. D: p53 mutation status. E: Metastasis in COAD (the first layer asterisk above the error bar represents a comparison to the normal group (p<0.001) compared to the normal group). F: The expression levels of LDHB were also validated through the Protein Atlas database.

negatively (n=100) correlated genes of LDHB (Table 1). Most genes were mainly enriched in metabolic processes, biological regulation, and cellular component organization; located in the nucleus; and function in protein and nucleic binding activity. Using WebGestalt, it was also found that most genes are mainly augmented in DNA replication and cell cycle pathway. The protein-protein interaction network analysis revealed the presence of 920 nodes and 23766 edges, with an average node degree of 51.7. The average local clustering coefficient was found to be 0.463. The constructed PPI network complex comprised of 932 genes, and the PPI enrichment (p<0.001), as depicted in Figure 4A. The cytoHubba plugin was employed to identify the most significant target genes based on a confidence score greater than 0.4 and a maximum number of interactors exceeding 5. The top ten genes with the highest degree score were determined, including CDK1, CCNB1, CDC20, CCNA2, MAD2L1, AURKB, PLK1, TOP2A, AURKA, and BUB1B (Figure 4B). The top 10 genes are involved in various biological processes such as protein kinase activity (CDK1, AURKB, PLK1, AURKA, BUB1B), regulatory protein function (CCNB1, CDC20, CCNA2), enzyme catalysis (TOP2A), and protein-coding (MAD2L1) as presented in Table 2. Most of these genes were involved in the cell

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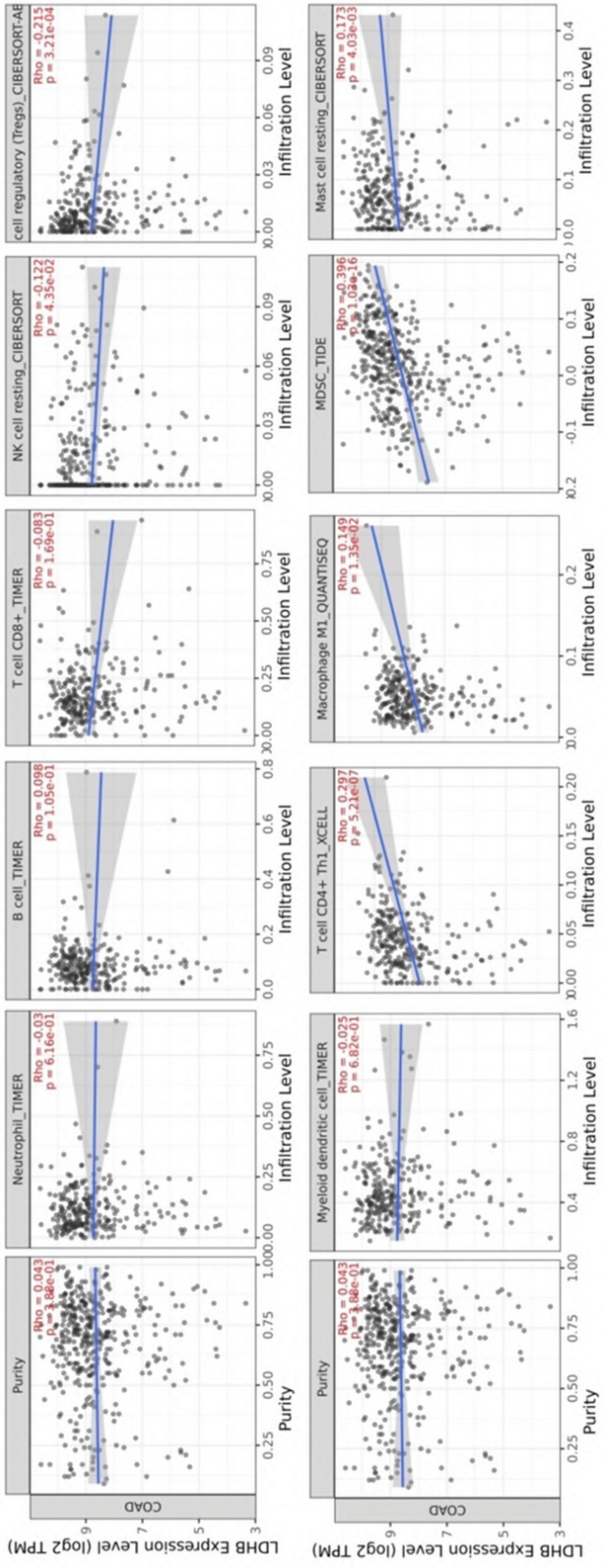

Figure 3. Correlation analysis between LDHB and immune infiltration level. The correlation of LDHB and tumor purity, LDHB and level of infiltrations from ten indicators (Neutrophil, B cells, $CD8^+$ T cells, NK cell, cell regulatory (Tregs), dendritic cell, $CD4^+$ T cells, macrophages, MDSC, and mast cell) were analyzed with TIMER in COAD.

Table 1. Gene ontology of LDHB correlated genes from DAVID 6.8.

Term	*p* -value	Count
Biological Process		
GO:0010467~gene expression	6,50E-21	378
GO:0034645~cellular macromolecule biosynthetic process	3,60E-13	335
GO:0016070~RNA metabolic process	1,10E-13	318
GO:0018130~heterocycle biosynthetic process	5,20E-05	261
GO:0019438~aromatic compound biosynthetic process	9,10E-05	260
Molecular Function		
GO:1901363~heterocyclic compound binding	7,38E-27	462
GO:0097159~organic cyclic compound binding	4,34E-25	462
GO:0003676~nucleic acid binding	9,01E-23	354
GO:0003723~RNA binding	3,21E-52	249
GO:0036094~small molecule binding	3,96E-12	241
Cellular Component		
GO:0005634~nucleus	3,45E-11	389
GO:0005654~nucleoplasm	1,60E-63	346
GO:0005737~cytoplasm	1,30E-13	342
GO:0005829~cytosol	3,88E-12	279
GO:0016020~membrane	4,80E-56	175
KEGG Pathway		
hsa01100:Metabolic pathways	4,80E-06	107
hsa01130:Biosynthesis of antibiotics	1,40E-08	36
hsa04110:Cell cycle	7,50E-15	35
hsa03013:RNA transport	4,50E-08	31
hsa03040:Spliceosome	3,50E-10	30

cycle process, particularly in the G2/M phase. The findings have demonstrated that the co-expression of LDHB plays a significant role in COAD.

Discussion

The causal relationship between LDHB and cancer is substantially more intricate than LDHA. A previous study reported that high LDHB level in lung adenocarcinoma is a significant prognostic factor for ameliorated survival rates.[13] In non-small cell lung cancer (NSCLC), inhibition of LDHB results in reduced tumor formation in xenograft tumors, which is facilitated by persistent mitochondrial DNA damage and reduced levels of mitochondria-dependent metabolites.[14] In contrast, hepatocellular carcinoma patient with low expression of LDHB predicts an unfavourable outcome in the patient.[15] At the same time, LDHB levels in breast cancer have been identified as a marker for evaluating the response to neoadjuvant treatment.[16] LDHB was found to be related to immune infiltration $CD4^+$, macrophage, and mast cells. Furthermore, the co-expression of LDHB was related to numerous critical vital genes in the advancement of cell cycles, particularly in the G2/M phase. These findings may offer early evidence for the role of LDHB in COAD carcinogenesis.

Despite TP53 mutation cases, LDHB expression in COAD was constantly high (Figure 2D). Recent mechanistic studies reported that p53 interacts with LDHB as a binding partner.[17] These associations adjust LDH complex composition and enzymatic activity, favouring pyruvate synthesis beyond lactate production. p53 inhibits LDHB activity[17], inhibiting glycolysis and glucose uptake by redirecting glucose to oxidative mitochondrial metabolism and fatty acid breakdown.[17,18] This may also be linked with p53, other metabolic functions inhibiting glucose-6-phosphatase dehydrogenase (G6PD) enzymatic activity.[17] However, it remains to be explored how COAD-associated-p53 mutation capability regulates LDH activity. If so, the p53-mutant could promote therapeutical vulnerability by interrupting glycolysis and glucose oxidation.

In this study, we uncover the constantly high expression of LDHB in several COAD sample types, including adenocarcinoma, mucinous adenocarcinoma, TP53 mutant, COAD stages 1–4, metastasis in no regional lymph node (N1), and metastasis in up to nine axillary

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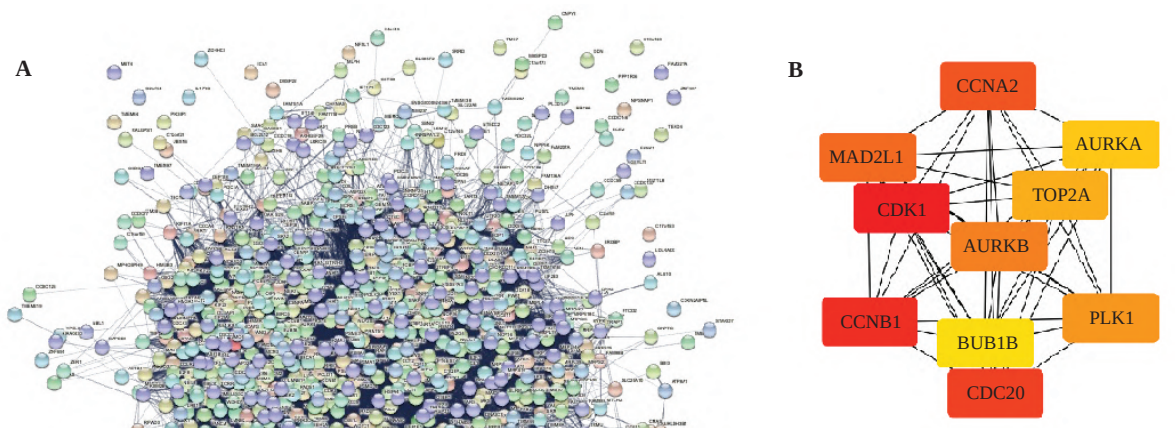

Figure 4. Protein-protein interaction network complex and top genes with the highest degree score. A: Protein-protein interaction of positively and negatively correlated genes of LDHB. B: Protein-protein interaction of top 10 hub gene.

lymph nodes (N1 and N2) (Figure 2A-2E). These findings confirm that an enhancement in lactate metabolism, notably LDHB, occurs from the initiation of cancer through its long-term stages, metastasis, and invasion.[19] In the COAD stages and metastasis nodal sample types (Figure 2C and 2E), LDHB expression tends to be regularly high because lactate metabolism in cancer cells occurs throughout carcinogenesis as a result of dramatically increase of glucose and lactate uptake in cancer formation.[19] In addition, tough LDHB expression in both adenocarcinoma and mucinous adenocarcinoma displayed an equal TPM, recent studies reported that COAD with abundance mucin or mucinous adenocarcinoma tend to demonstrate poor prognosis compared with those adenocarcinomas.[20] This such a challenge to explore the correlation of mucin over-production and lactate metabolism in COAD, particularly mucin 2 (MUC2) and mucin 5AC (MUC5AC) proteins, with LDHB.

The expression status of LDHB possessed an uncertainly metabolic marker among all cancer types though numerous studies mentioned its contribution to cancer development and progression.[21] There is evidence to suggest that overexpression of LDHB in COAD may have a negative impact on survival rates. This is because lactate, which is produced as a result of TME adaptation, has been linked to wound healing, chronic inflammation, and cancer development.[9,22] The exact mechanism is not clearly understood, but a change in LDHB expressions often alters cancer cells' metabolic adaptability in controlling lysosomal activity for cell proliferation and maintaining energy.[22] In

Table 2. The role and function of LDHB correlated genes.

Protein	Role	Function	References No.
CDK1	Protein Kinase	G2/M regulator	33
CCNB1	Subunit Regulator CDK1	Control of the cell cycle at the G2/M (mitosis) transition	34
CDC20	Regulatory Protein	Activate the APC and chromosome separation	35
CCNA2	Regulatory Protein	Activate CDK2 and promotes G1/S and G2/M transition	36
MAD2L1	Protein Coding	Mitotic spindle assembly checkpoint (anaphase)	25
AURKB	Protein Kinase	Chromosomemicrotubule attachment	37
PLK1	Protein Kinase	Chromosome segregation, spindle assembly, and cytokinesis	28
TOP2A	Enzyme Catalyst	Chromosome separation and DNA replication	38
AURKA	Protein Kinase	Chromosomemicrotubule attachment	39
BUB1B	Protein Kinase	Spindle checkpoint	40

brief, LDHB helps cancer cells to use lactate over glucose to maintain the process of autophagy. LDHB-mediated autophagy via SIRT5-deacetylation in colorectal cancer was a key event in tumorigenesis.[23] The latest study reported an inhibition of tumor growth in COAD by interrupting the glycolysis process promoted by suppressing Hypoxia-inducible factor 1 (HIF1-a) and LDHB by Ribosomal protein S7 (RPS7).[24] Next, the newest study reported that the downregulation of LDHB expression by KLF14 showed improved overall survival in late-stage COAD patients.[21] In addition, LDHB was found to be closely related to glycolysis via the KRAS pathway[13], in which KRAS mutation promotes the initiation and progression of cancers, including COAD. In lung adenocarcinoma with KRAS mutation, LDHB was associated with tumor malignancy.[13] Consequently, further research is required to determine LDHB's involvement in COAD.

The gene co-expression structure attentively displays the molecular relationship and physiologically corresponds to targeted genes with biological processes, expanding information mining in molecular function and clinical application value. Correlated genes of LDHB are associated with binding function and biological processes in terms of gene synthesis and most biosynthetic and metabolic processes (Table 1). Under KEGG pathway computation, these genes primarily function in DNA replication, cell cycle, and cellular metabolism. These results were consistent with the gene ontology of LDHB itself.[19] We found the top 10 genes that are mainly crucial in cell proliferation, especially in malignant cell cycle progression, that influence the metastasis and prognosis of COAD. MAD2L1 is related to the LDH-mediated Warburg effect in gastric cancer.[25] Moreover, the central regulation in metabolic response, HIF1-a[26], is essential in cell cycle arrest by its association with CDK1, CDK2, p21, and p27[27]. Polo-like kinases (PLKs), including PLK1, have also been reported to regulate metabolic processes in colorectal cancer by lowering the glycolytic rate.[28] Interestingly, the loss function of aurora kinases (AURKA and AURKB) resulted in a more significant reduction of cellular viability in glioblastoma (GBM) in typical glucose conditions.[29] The involvement of cell cycle regulators in the cancer metabolic process and their canonical function controlling the cell cycle process has been studied in recent years. The possibility of targeting cancer metabolism using cell cycle inhibitor seem increasingly challenging. A detailed examination of the regulation of LDHB-associated genes in metabolic reprogramming is an interdependent feature that emphasizes the need for metabolic control for cell survival. Multiple types of immune cell infiltrations have been linked to LDHB expression levels in cancer malignancies. Increased LDHB expression may be associated with adverse outcomes in COAD patients. This may be given due to the effects of lactic acid accumulation on immune surveillance and intracellular cytokine secretion, which may hinder the efficacy of certain treatments, such as adoptive T cell and checkpoint inhibition therapy.[30,31] Recent studies demonstrate the involvement of LDHB overexpression in promoting $CD4^+$ and $CD8^+$ infiltration, resulting in TME reorganizing in HCT116 colorectal cancer cells.[31] In addition, LDHB-knocked cells showed to inhibit the activity of HIF1-a, accompanied by increasing infiltration of $CD4^+$ and $CD8^+$ T cells.[32] Briefly, dysfunction of LDHB inhibits tumor progression by mediating the aggregation of T cells and NK cells, thereby preventing tumor immune evasion. LDHB expression levels was identified as an important factor affecting cetuximab sensitivity, in which upregulated LDHB correlated with the acquisition of resistance in cetuximab therapy.[5] A recent bioinformatic study using GSE40967 datasets reported that COAD patients with higher lactate metabolism may acquire an immunosuppressive tumor microenvironment, which subsequently led to immune escapes with infiltration of $CD4^+$ T cells and dendritic cells, resulting in poor prognoses.[9] A recently published retrospective study demonstrates that serum lactate dehydrogenase-to-albumin ratio could be an important marker to predict the overall survival of COAD patients.[22] From those findings, a deeper understanding of LDHB in cancer would promote a practical and inexpensive marker to predict the prognosis of many cancers, including COAD. As part of our investigation, we utilized the TIMER tool to perform an Immune Correlation analysis to examine the levels of LDHB expression and immune infiltration in COAD. $CD4^+$ T cell, MDSC, macrophage, and mast cell infiltration correlated with LDHB expression. Our findings could confirm that LDHB is associated with immune response in COAD, suggesting that LDHB could be developed as a novel target for combating immune tolerance and escape. However, more than these data are needed to explain the precise role of LDHB in tumor immune regulation; additional research and laboratory experiment are required to establish these preliminary findings. Deepening bioinformatic exploration using datasets from the Gene Expression Omnibus (GEO) database could also be done to assess the importance of LDHB in specific conditions of COAD patients.

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Conclusion

Overall, LDHB was highly expressed in all sample types of COAD and correlated with poor prognosis. LDHB expression may also affect COAD carcinogenesis by increasing immune-infiltrating $CD4^+$, $CD8^+$ T cells, macrophages, MDSCs, and mast cells. More specifically, the LDHB-related genes show significance in the cell cycle process, which may have a function in the metabolic process. In conclusion, LDHB may be a potential cell cycle target and immunological checkpoint for COAD diagnosis and therapy.

Authors Contribution

FW and MH performed data collection, analysis, wrote the paper, and participated in the final edition of the manuscript. FW performed data validation and contributed the main idea of the work. All authors discussed the results and commented on the manuscript.

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