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The effect of glycolytic enzyme expression and thyroiditis on the aggressiveness of papillary thyroid carcinoma: a retrospective cohort study

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Purpose: Glycolytic enzymes have been extensively studied in various cancer types, revealing their aggressive characteristics and roles in tumor progression. This study aimed to determine whether the expression of glycolytic enzymes is associated with aggressiveness in the presence or absence of chronic lymphocytic thyroiditis (CLT).
Methods: The expression of hexokinase 2, lactate dehydrogenase A (LDHA), pyruvate kinase isoform M2 (PKM2), glucose transporter 1 (GLUT1), and monocarboxylate transporter 4 (MCT4) was examined in 233 papillary thyroid carcinoma (PTC) specimens by immunohistochemistry. We evaluated whether the expression of these glycolytic enzymes correlates with lymph node metastasis, extrathyroidal extension (ETE), and recurrence rate, both with and without CLT. In addition, we analyzed the correlation between glycolytic enzyme messenger RNA expression and risk factors in PTC using The Cancer Genome Atlas.
Results: All glycolytic enzymes and transporter proteins were overexpressed in PTC compared with normal tissue. PKM2 expression was most highly correlated with the other glycolytic enzymes. High PKM2 expression was significantly linked to increased recurrence risk in patients without CLT (hazard ratio, 1.76; 95% confidence interval, 1.01–3.06; P = 0.046), but this association was not observed in those with CLT.
Conclusion: Overexpression of LDHA, PKM2, GLUT1, and MCT4 is associated with PTC. CLT is significantly associated with an increased incidence of gross ETE and, paradoxically, with a reduced recurrence rate in PTC. LDHA expression was lower in the presence of CLT, whereas PKM2 remained consistently linked to a higher recurrence rate in its absence. Among the evaluated glycolytic enzymes, PKM2 may serve as a biomarker for recurrence in PTC.
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Key Words: Chronic lymphocytic thyroiditis, Glycolytic enzyme, Thyroid neoplasms, Warburg effect

INTRODUCTION

Papillary thyroid carcinoma (PTC) generally has a favorable prognosis; however, approximately 40%–90% of cases demonstrate metastatic potential, particularly involving the lymph nodes [1]. One major factor contributing to a poorer prognosis in PTC is the presence of the BRAF mutation, which has been associated with increased lymph node metastasis (LNM), recurrence, and cancer-related mortality [2-4]. Although testing for the BRAF mutation aids in diagnosis, its value as

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an independent prognostic marker remains inconsistent, especially in populations with a high mutation prevalence, such as Koreans [3,5-7]. Additional markers are therefore needed to more reliably predict aggressiveness in PTC.

Recent research has highlighted the role of key glycolytic enzymes, including pyruvate kinase isoform M2 (PKM2) and lactate dehydrogenase A (LDHA), in tumor metabolism. These enzymes support cancer cell proliferation through enhanced glucose uptake and lactate production, even under aerobic conditions—a phenomenon known as the Warburg effect [3,8-12]. They are frequently overexpressed in aggressive malignancies, including PTC, and have been linked to increased invasiveness and unfavorable clinical outcomes [13,14]. In particular, PKM2 has been shown to be highly expressed in cancer-associated fibroblasts within the tumor microenvironment, thereby driving glucose uptake and lactic acid production. These metabolic changes promote cellular movement and invasiveness [15-19].

Lactate accumulation in the tumor microenvironment, primarily facilitated by monocarboxylate transporter 4 (MCT4), leads to extracellular acidification that supports tumor growth, invasion, and immune evasion [20,21]. High levels of LDHA, often associated with the BRAF mutation, further enhance aggressiveness in PTC by promoting aerobic glycolysis. Preliminary studies have demonstrated that LDHA expression correlates with BRAF mutational status, suggesting its potential as a prognostic biomarker [22,23].

In studies comparing recurrence rates and survival outcomes based on glycolytic enzyme expression across different thyroid cancer types, findings have indicated significant associations between enzyme overexpression and poor prognosis [24-26]. In medullary thyroid carcinoma, HK2 and MCT4 protein overexpression has been linked to lower survival rates. In poorly differentiated thyroid carcinoma, MCT4 and glucose transporter 1 (GLUT1) protein overexpression have been associated with reduced survival rates. These findings suggest that the glycolytic shift may contribute to tumor aggressiveness and poor clinical outcomes beyond PTC [24]. In an anaplastic thyroid carcinoma (ATC) cell line study, MCT4 inhibition was reported to suppress ATC growth [25]. Another ATC cell line study reported that PKM2 plays a role in enhancing metastasis and promoting aerobic glycolysis [26].

In one study, a significant increase in glycolytic enzymes such as GLUT1, HK2, PKM2, and LDHA was observed in thyroid inflammation compared to the normal control group, which was associated with an increased extracellular acidification rate and oxygen consumption rate. These findings suggest that thyroid inflammation may play a significant role in shaping the peritumoral microenvironment [27].

In a previous study, we examined whether glycolysis was increased in PTC by comparing glycolytic enzyme expression in cancerous versus matched normal thyroid tissues using immunohistochemistry (IHC). Patients with chronic lymphocytic thyroiditis (CLT) were excluded from that prior work. We subsequently compared glycolytic enzyme expression according to BRAF mutational status. We found that LDHA levels were significantly higher in the BRAF mutation group, whereas PKM2 and GLUT1 levels did not differ significantly between the BRAF-mutated and BRAF wild-type groups. Additionally, we investigated whether the BRAF mutation upregulates LDHA expression via activation of the mitogen-activated protein kinase signaling pathway in human thyroid cell lines, as well as whether inhibiting BRAF reduces LDHA expression in these cell lines. Furthermore, we indirectly confirmed that high LDHA messenger RNA (mRNA) expression is associated with PTC aggressiveness using data from The Cancer Genome Atlas (TCGA) thyroid cancer database [22].

The present study investigates the correlation between the expression of glycolytic enzymes, including LDHA and PKM2, and PTC aggressiveness such as LNM and extrathyroidal extension (ETE) according to the presence or absence of CLT. Our goal is to improve prognostic accuracy and inform clinical decision-making for patients with PTC.

METHODS

Ethics statement

The study protocol was approved by the Institutional Review Board of Yonsei University College of Medicine (No. 4-2022-0593), which waived the requirement for informed consent.

Patients

From January 2013 to December 2016, a total of 233 patients who underwent thyroid surgery and were preoperatively diagnosed with PTC by fine needle aspiration at Yonsei University Severance Hospital were enrolled. Inclusion and exclusion criteria were applied to ensure that only patients with available pathological results were included. All samples were obtained as formalin-fixed, paraffin-embedded tissue blocks.

The inclusion criteria were as follows: (1) age ≥20 and <70 years and (2) PTC with tumor size ≥1 cm. The exclusion criteria were as follows: (1) age <20 or ≥70 years; (2) papillary thyroid microcarcinoma with tumor size <1 cm; (3) follicular, medullary, or anaplastic carcinoma; (4) distant metastasis; (5) insufficient histopathological results; and (6) history of radiation exposure.

Propensity score matching process

Propensity score matching was used to create comparable groups of patients with and without recurrence, matching them in a 1:3 ratio based on age, sex, tumor size, ETE, multifocality, and cervical LNM. Patients with similar propensity scores—

representing comparable probabilities of recurrence—were matched to ensure balanced distributions of these 6 baseline covariates between the groups. After matching, 7 patients were excluded due to a lack of formalin-fixed, paraffin-embedded tissue blocks.

Preparation of tissue microarray

A 2-mm-diameter tissue microarray (TMA) apparatus (TMA set, Labro) was used. Paraffin blocks containing vertically embedded thyroid tissue were carefully punched to obtain 2-mm-diameter, 5-mm-long paraffin cores. These cores, each containing representative tumor or normal thyroid tissue from a single patient, were embedded into a new paraffin block approximately 5–10 mm thick. A limitation of the TMA method is the potential heterogeneity in the distribution of thyroiditis, which may not be consistently captured during core sampling. To mitigate this issue, a pathologist carefully reviewed representative pathology slides to ensure that both tumor and peritumoral normal tissue were accurately represented in the selection process. The premanufactured plastic TMA cassette (2-mm lumen-sized TMA cassette, Labro) contained 30 sockets (2 mm in diameter each). Spacing between transplanted tissues was maintained at 0.5 mm, allowing up to 30 tissue sections on a single slide.

If a tissue core was not properly aligned in the paraffin block, the angle of the block was adjusted. After placing the paraffin cores into the cassette, it was immersed in molten paraffin (at approximately 65 °C) within a metal mold designed for the TMA cassette. The mold was then placed on a hot plate for 5 minutes, allowing the paraffin tissue columns and the molten paraffin to fuse. Next, the mold was placed on a cold plate to solidify, creating a stable TMA block (Fig. 1).

Immunohistochemistry

Paraffin-embedded tissue specimens were cut into 4-μm-thick sections. IHC staining was performed using a Discovery XT autoimmunostainer (750-701, Ventana) with monoclonal or polyclonal antibodies against GLUT1 (dilution 1:100, Catalog No. RM0063, Medaysis), PKM2 (dilution 1:100, Catalog No. AF5234, Affinity), LDHA (dilution 1:400, Catalog No. DF6280; Affinity), hexokinase 2 (HK2; dilution 1:1,000, Catalog No. BF0283, Affinity), and MCT4 (SLC16A4; dilution 1:100, Catalog No. DF7145, Affinity) according to the manufacturer's instructions.

IHC results were assessed by an expert using an immunoreactive score (IRS) derived by multiplying the intensity of staining (I) (0–3 points: absent, weak, moderate, and strong, respectively) by the percentage of positively stained tumor cells (P) (0–4 points: 0%, 1%–10%, 11%–49%, 50%–80%, and 80%–100%, respectively). The final IRS ($H = P \times I$) was evaluated as low (0–4 points), moderate (6–8 points), or high (9–12 points). For risk factor analysis, the IRS was divided into low (0–4 points) and high (5–12 points) categories (Fig. 2).

Data from The Cancer Genome Atlas thyroid cancer database

To investigate these associations in PTC, we used the TCGA dataset. Of the initial 507 thyroid cancer cases, we excluded patients with other malignancies or missing data on BRAF, LDHA, PKM2, and GLUT1 expression. Publicly available mRNA sequencing data, somatic mutation data, and clinical information from 465 patients with thyroid cancer were obtained from TCGA (version 2016_01_28; https://gdac.broadinstitute.org). Overall survival, disease-free survival (DFS), disease-specific survival, and progression-free survival data were downloaded from cBioPortal (TCGA, Firehose Legacy, and PanCancer Atlas). All data were fully anonymized prior to access. Thyroid cancer staging was based on the 7th edition of the American Joint Committee on Cancer staging system.

Primary outcomes assessment

This study examined whether glycolytic enzyme expression correlates with aggressiveness and oncological outcomes, such as recurrence, in intermediate-risk PTC larger than 1 cm. Furthermore, we evaluated whether glycolytic enzyme expression is associated with LNM, ETE, and recurrence in the presence or absence of CLT, a condition characterized by pathological lymphocytic infiltration of thyroid tissue and the presence of anti-thyroglobulin antibodies. In addition, this study aimed to evaluate whether glycolytic enzyme expression is correlated with aggressiveness, such as LNM and recurrence, using TCGA data.

Statistical analysis

The Student t-test was used to assess differences in continuous variables between groups. The chi-square test or Fisher exact test was used to compare categorical variables. Continuous variables are reported as the mean ± standard deviation with ranges, and categorical variables are expressed as percentages and absolute numbers. Univariate and multivariate analyses were performed to identify variables independently associated with recurrence, and odds ratios (ORs) with 95% confidence intervals (CIs) were calculated. Differences with $P < 0.05$ were considered statistically significant.

Univariate and multivariate Cox proportional hazards modeling were conducted to assess the association between glycolytic enzyme expression and PTC aggressiveness. Hazard ratios (HRs) with 95% CIs were calculated. Statistical analyses were performed using IBM SPSS Statistics for Windows, ver. 21.0 (IBM Corp.), with statistical significance defined as $P < 0.05$.

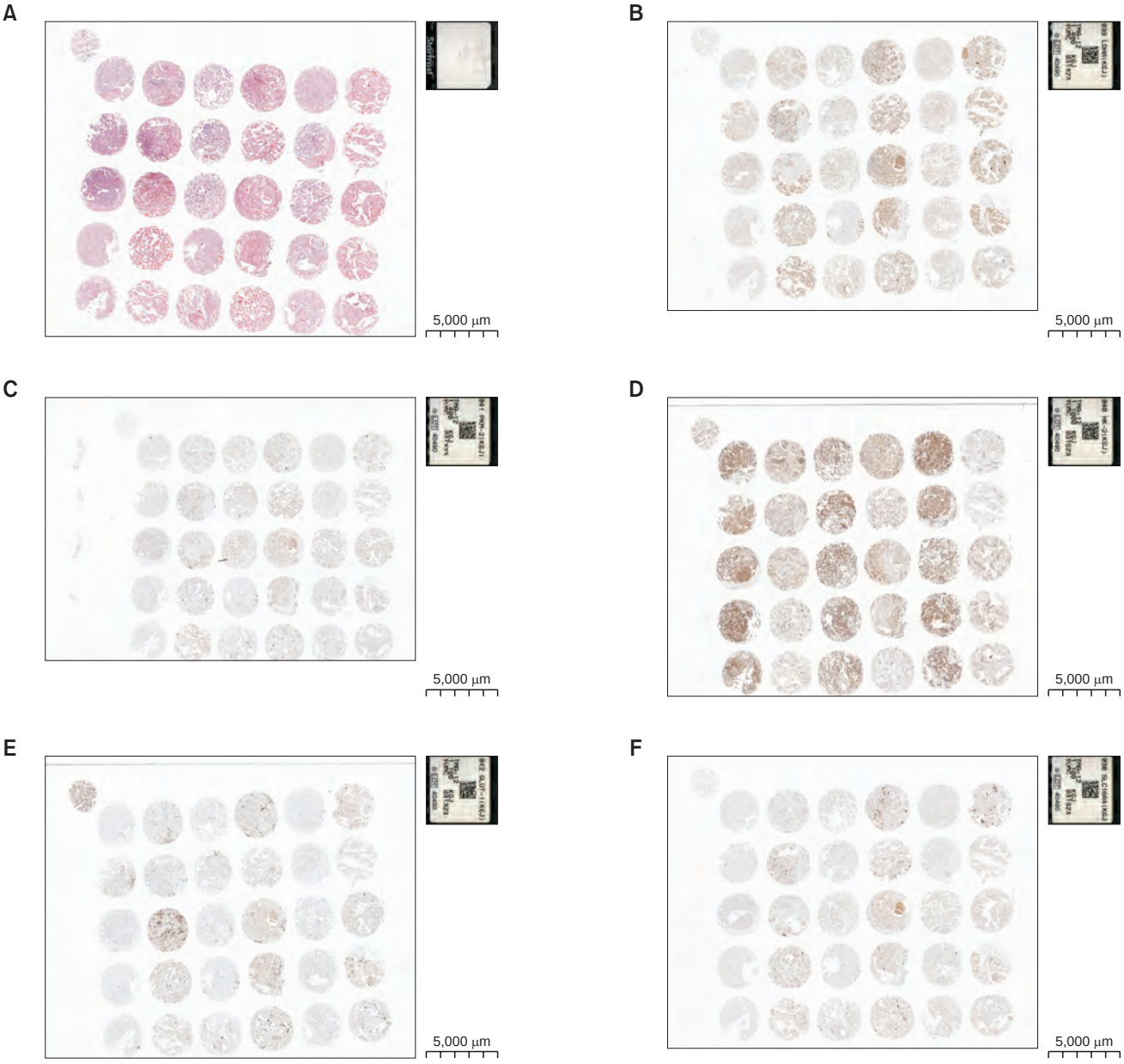

Fig. 1. Immunohistochemistry staining on tissue microarray comprising 15 paired samples of cancerous and normal tissues on a single slide. (A) H&E stain, (B) lactate dehydrogenase A, (C) pyruvate kinase isoform M2, (D) hexokinase 2, (E) glucose transporter 1, and (F) monocarboxylate transporter 4.

RESULTS

Expression of glycolytic enzymes in human thyroid cancer tissues

We assessed the expression of glycolytic enzymes and associated transporters in TMAs, comparing normal thyroid tissue with cancerous tissue. HK2, PKM2, LDHA, and GLUT1 were all significantly elevated in tumor tissues. Although MCT4 levels also increased, this elevation was less pronounced than that observed for the other enzymes. These results indicate a prominent upregulation of glycolytic pathways in thyroid cancer (Table 1).

Given the observed differences in glycolytic enzyme expression, we subsequently explored the correlations among these enzymes to better understand their interactions within the glycolysis pathway.

Correlation between enzymes in the glycolysis pathway

LDHA maintains the Warburg effect by converting pyruvate to lactate. PKM2 regulates pyruvate production, influences glycolytic flux, and activates the HIF-1α pathway, which in turn

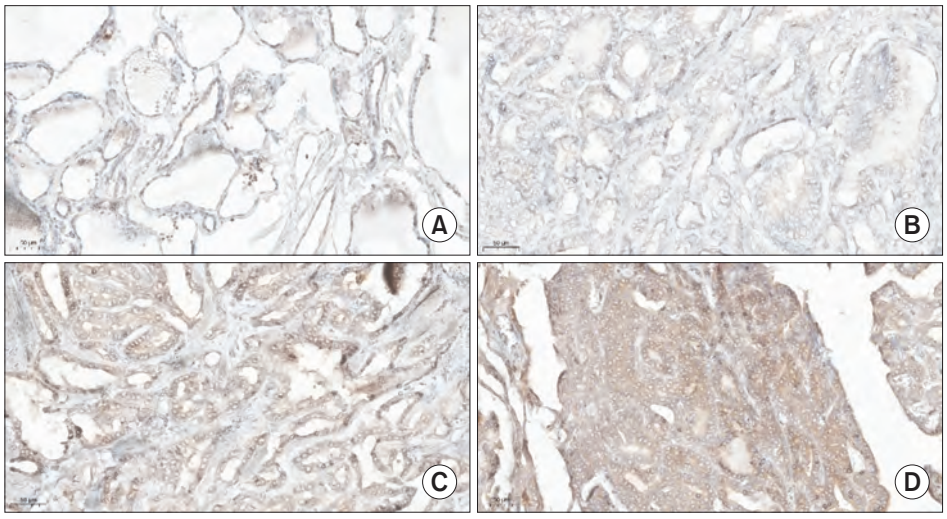

Fig. 2. Immunohistochemical analysis of pyruvate kinase isoform M2 expression in normal thyroid and papillary thyroid carcinoma tissues (×400 magnification). (A) Normal thyroid tissue; papillary thyroid carcinoma with (B) weak staining, (C) intermediate staining, and (D) strong staining.

Table 1. Glycolytic enzymes and transporter proteins expression level in normal and papillary thyroid carcinoma tissues

Variable	Normal (n = 233)	Tumor (n = 233)	P-value
HK2 expression			<0.001
Low	3	0	
Moderate	54	0	
High	176	233	
PKM2 expression			<0.001
Low	233	178	
Moderate	0	46	
High	0	9	
LDHA expression			<0.001
Low	230	124	
Moderate	3	58	
High	0	51	
GLUT1 expression			<0.001
Low	233	181	
Moderate	0	42	
High	0	10	
MCT4 expression			0.008
Low	232	221	
Moderate	1	11	
High	0	1	

HK2, hexokinase 2; PKM2, pyruvate kinase isoform M2; LDHA, lactate dehydrogenase A; GLUT1, glucose transporter 1; MCT4, monocarboxylate transporter 4.

affects the expression of LDHA and MCT4. MCT4 enhances the external release of lactate produced by LDHA, thereby shaping a microenvironment conducive to cancer cell survival. Overall, increases in enzyme levels were observed concurrently, except in the case of HK2. Among these enzymes, PKM2 displayed the strongest correlations with the others, including a particularly robust correlation with MCT4 (r = 0.613). This suggests that

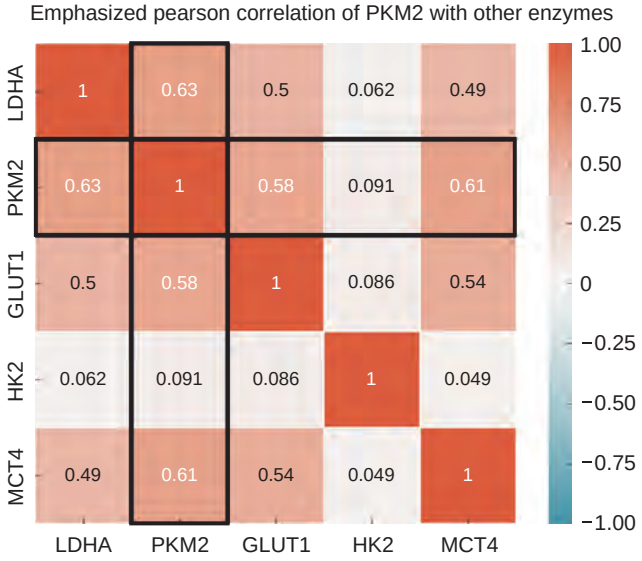

Fig. 3. Correlation among glycolytic enzyme expression levels in papillary thyroid carcinoma. PKM2, pyruvate kinase isoform M2; LDHA, lactate dehydrogenase A; mRNA, messenger RNA; GLUT1, glucose transporter 1; MCT4, monocarboxylate transporter 4.

pyruvate processing and lactate transport are closely linked, underscoring the central role of PKM2 in cancer metabolism and the Warburg effect (Fig. 3).

Clinical characteristics based on pyruvate kinase isoform M2 expression levels

We compared the clinicopathological characteristics of PTC patients with low (n = 178) and high (n = 55) PKM2 expression. No significant differences were found between the 2 groups in terms of age, sex, tumor size, ETE, multifocality, lymphovascular invasion, BRAF mutation, or TNM staging. LDHA, GLUT1, and MCT4 were significantly elevated in the

Table 2. Clinicopathological characteristics of patients with papillary thyroid carcinoma according to PKM2 expression level

Characteristic	PKM2 expression level		P-value
	Low	High	
No. of patients	178	55	
Age (yr)	45.5 ± 13.5	43.4 ± 14.4	0.338
Sex			0.682
Male	57 (32.0)	16 (29.1)	
Female	121 (68.0)	39 (70.9)	
Tumor size (cm)	1.75 ± 0.99	1.91 ± 1.84	0.398
Extrathyroidal extension			0.163
Negative	33 (18.5)	16 (29.1)	
Minimal	117 (65.7)	34 (61.8)	
Gross	28 (15.7)	5 (9.1)	
Multifocality			0.360
Negative	104 (58.4)	38 (69.1)	
Unilateral	20 (11.2)	5 (9.1)	
Bilateral	54 (30.3)	12 (21.8)	
Lymphovascular invasion			0.080
No	35 (19.7)	17 (30.9)	
Yes	143 (80.3)	38 (69.1)	
Thyroiditis			0.674
No	141 (79.2)	45 (81.8)	
Yes	37 (20.8)	10 (18.2)	
Clinical N stage			0.904
cN0	124 (69.7)	38 (69.1)	
cN1a	19 (10.7)	5 (9.1)	
cN1b	35 (19.7)	12 (21.8)	
T stage AJCC8th			0.107
T1	119 (66.9)	37 (67.3)	
T2	20 (11.2)	11 (20)	
T3	24 (13.5)	2 (3.6)	
T4	15 (8.4)	5 (9.1)	
N stage			0.769
N0	64 (36)	20 (36.4)	
N1a	76 (42.7)	21 (38.2)	
N1b	38 (21.3)	14 (25.5)	
M stage			0.947
M0	175 (98.3)	54 (98.2)	
M1	3 (1.7)	1 (1.8)	
BRAF^{V600E} mutation			0.540
WT	10 (5.6)	4 (7.2)	
V600E	74 (41.6)	20 (36.4)	
Extent of surgery			0.269
Less than total	36 (20.2)	15 (27.3)	
Bilateral total thyroidectomy	142 (79.8)	40 (72.7)	
Recurrence			0.031*
No	139 (78.1)	35 (63.6)	
Yes	39 (21.9)	20 (36.4)	
Recurrence site			0.579
Regional lymph node	36 (90.0)	20 (95.2)	
Distant metastasis	4 (10.0)	1 (4.8)	
HK2 expression			0.999
Low	0 (0)	0 (0)	
High	178 (100)	55 (100)	
LDHA expression			0.001*
Low	115 (64.6)	9 (16.4)	
High	63 (35.4)	46 (83.6)	
GLUT1 expression			0.001*
Low	157 (88.2)	24 (43.6)	
High	21 (11.8)	31 (56.4)	
MCT4 expression			0.001*
Low	177 (99.4)	44 (80.0)	
High	1 (0.6)	11 (20.0)	

Values are presented as number only, mean ± standard deviation, or number (%).
PKM2, pyruvate kinase isoform M2; HK2, hexokinase 2; LDHA, lactate dehydrogenase A; GLUT1, glucose transporter 1; MCT4, monocarboxylate transporter 4.
*P < 0.05.

PKM2 high group (P = 0.001), except for HK2. However, patients with high PKM2 expression had a significantly higher recurrence rate (36.4%) than those with low PKM2 expression (21.9%) (P = 0.031). Recurrences were predominantly regional lymph node events in both groups, and the recurrence sites did not differ significantly (P = 0.579) (Table 2). Thus, although PKM2 expression did not correlate with most clinicopathological factors, it was clearly associated with an increased risk of recurrence in PTC patients.

Having established that PKM2 correlates with recurrence, we then sought to identify additional factors contributing to direct invasion, a hallmark of aggressive thyroid cancer.

Glycolytic enzymes expression in papillary thyroid carcinoma with and without thyroiditis

We assessed the expression of glycolytic enzymes and associated transporters in TMAs, comparing with thyroiditis and without thyroiditis. High LDHA expression was more prevalent in the thyroiditis-negative group (52.7%) compared to the thyroiditis-positive group (23.4%). Other glycolytic enzymes (HK2, PKM2, GLUT1, MCT4) showed no significant differences based on thyroiditis status (Table 3).

Risk factors for direct invasion

Older age (≥55 years), the presence of thyroiditis, and larger tumor size were significantly associated with an increased risk of gross ETE in both univariate and multivariate analyses. Age and thyroiditis were particularly influential, with ORs of 4.73 (P < 0.001) and 4.42 (P < 0.001), respectively. Other factors, including sex and the expression levels of LDHA, GLUT1, and PKM2, showed no significant association with gross ETE (Table 4).

Risk factors for clinical lymph node metastasis

In univariate analysis, male sex, multifocality, bilateral tumors, minimal and gross ETE, and larger tumor size were significantly associated with an increased risk of clinical LNM. Following multivariate analysis, gross ETE emerged as the most robust independent predictor of LNM, while minimal ETE lost significance after adjusting for other factors. Elevated LDHA, GLUT1, PKM2, and MCT4 expression levels were not significantly linked to LNM in these analyses (Table 5).

Table 3. Glycolytic enzyme and transporter expression levels according to thyroiditis

Variable	Thyroiditis (–) (n = 186)	Thyroiditis (+) (n = 47)	P-value
HK2 expression			0.999
Low	0 (0)	0 (0)	
High	186 (100)	47 (100)	
PKM2 expression			0.674
Low	141 (75.8)	37 (78.7)	
High	45 (24.2)	10 (21.3)	
LDHA expression			0.001*
Low	88 (47.3)	36 (76.6)	
High	98 (52.7)	11 (23.4)	
GLUT1 expression			0.325
Low	147 (79.0)	34 (72.3)	
High	39 (21.0)	13 (27.7)	
MCT4 expression			0.132
Low	174 (93.5)	47 (100)	
High	12 (6.5)	0 (0)	

Values are presented as number (%).
HK2, hexokinase 2; PKM2, pyruvate kinase isoform M2; LDHA, lactate dehydrogenase A; GLUT1, glucose transporter 1; MCT4, monocarboxylate transporter 4.
*P < 0.05.

Risk factors for recurrence

Both univariate and multivariate analyses identified male sex and high PKM2 expression as significant independent predictors of recurrence. Although larger tumor size was significant in univariate analysis, it did not maintain significance after adjustment. Other variables, including multifocality, ETE, and the expression of LDHA, GLUT1, MCT4, and HK2, were not significantly associated with recurrence. High PKM2 expression (OR, 2.05; P = 0.036) and LNM (OR, 3.90; P = 0.001) were linked to increased recurrence risk, whereas thyroiditis was associated with a reduced recurrence risk (OR, 0.40; P = 0.043) (Table 6, Fig. 4).

Cox regression analysis of DFS based on glycolytic enzyme expression and the presence of CLT revealed distinct prognostic implications. High PKM2 expression was significantly associated with an increased risk of recurrence in patients without CLT (HR, 1.76; 95% CI, 1.01–3.06; P = 0.046), whereas this association was not observed in those with CLT (HR, 0.20; 95% CI, 0.02–2.31; P = 0.197). LDHA and GLUT1 expression did not show a significant impact on DFS, regardless of CLT status. These findings suggest that PKM2 expression may be a potential prognostic marker in the absence of CLT, while the presence of CLT may mitigate its impact on disease recurrence (Fig. 5).

Peritumoral microenvironment and cancer recurrence in relation to thyroiditis

Given the apparent link between thyroiditis and recurrence, we further investigated how the peritumoral microenvironment, particularly in the presence of CLT, influenced recurrence. In cancer tissues from patients who experienced recurrence, the IRS values of LDHA, PKM2, and HK2 were similar regardless of CLT status. In cancer tissue from a patient who did not experience recurrence, the IRS values of

Table 4. Risk factors for gross extrathyroidal extension invasion in papillary thyroid carcinoma

Variable	Univariate		Multivariate	
	OR (95% CI)	P-value	OR (95% CI)	P-value
Age, *vs*. <55 yr	3.34 (1.52–7.33)	0.003	4.73 (1.96–1.41)	0.001*
Male sex	1.24 (0.56–2.75)	0.593		
Multifocality	1.57 (1.04–2.35)	0.031	1.47 (0.95–2.27)	0.085
Thyroiditis	2.54 (1.12–5.75)	0.026	4.42 (1.74–11.23)	0.002*
Tumor size	1.65 (1.20–2.26)	0.002	1.84 (1.30–2.61)	0.001*
BRAF^{V600E} mutation	1.21 (0.14 –10.48)	0.863		
LDHA high, *vs*. low	1.25 (0.59–2.67)	0.563		
GLUT1 high, *vs*. low	0.81 (0.32–2.10)	0.671		
PKM2 high, *vs*. low	0.59 (0.21–1.60)	0.297		

OR, odds ratio; CI, confidence interval; LDHA, lactate dehydrogenase A; GLUT1, glucose transporter 1; PKM2, pyruvate kinase isoform M2; MCT4, monocarboxylate transporter 4.
*P < 0.05.

Table 5. Risk factors for lymph node metastasis in papillary thyroid carcinoma

Variable	Univariate		Multivariate	
	OR (95% CI)	P-value	OR (95% CI)	P-value
Age, *vs*. ≥55 yr	1.94 (1.04–3.60)	0.036	2.33 (1.19–4.56)	0.013*
Male sex	2.37 (1.27–4.44)	0.007	2.53 (1.30–4.92)	0.006*
Multifocality	1.53 (0.60–3.91)	0.373		
Bilateral	1.04 (0.57–1.91)	0.894		
Thyroiditis	1.42 (0.71–2.84)	0.318		
Gross ETE, *vs*. minimal ETE and negative	2.60 (1.39–4.89)	0.003	1.13 (0.39–3.27)	0.824
Tumor size	1.09 (0.86–1.38)	0.460		
T stage				
T1	1		1	
T2	4.6 (0.47–44.60)	0.188	2.81 (0.27–28.70)	0.384
T3	2.07 (1.05–4.07)	0.036	1.93 (0.96–3.88)	0.064
T4	21.85 (2.68–178.12)	0.004	23.87 (2.85–199.64)	0.003*
BRAF^{V600E} mutation	1.03 (0.32–3.32)	0.965		
LDHA high, *vs*. low	0.65 (0.38–1.12)	0.120	0.72 (0.41–1.27)	0.259
GLUT1 high, *vs*. low	0.88 (0.46–1.65)	0.681		
PKM2 high, *vs*. low	0.98 (0.52–1.84)	0.956		
MCT4 high, *vs*. low	1.14 (0.33–3.89)	0.841		

OR, odds ratio; CI, confidence interval; ETE, extrathyroidal extension; LDHA, lactate dehydrogenase A; GLUT1, glucose transporter 1; PKM2, pyruvate kinase isoform M2; MCT4, monocarboxylate transporter 4.
*P < 0.05.

Table 6. Risk factors for recurrence in papillary thyroid carcinoma

Variable	Univariate		Multivariate	
	OR (95% CI)	P-value	OR (95% CI)	P-value
Age, *vs*. <55 yr	0.80 (0.39–1.65)	0.551		
Male sex	1.93 (1.04–3.57)	0.036	1.64 (0.86–3.13)	0.133
Multifocality	0.90 (0.64–1.26)	0.524		
Thyroiditis	0.45 (0.19–1.07)	0.071	0.40 (0.16–0.97)	0.043*
Extrathyroidal extension				
Negative or minimal	1		1	
Gross	1.49 (0.66–3.37)	0.342		
Tumor size	1.65 (1.01–2.70)	0.046	0.96 (0.75–1.24)	0.764
pN1	3.63 (1.72–7.63)	0.001	3.90 (1.83–8.31)	0.001*
BRAF^{V600E} mutation	0.72 (0.21-2.53)	0.607		
LDHA high, *vs*. low	1.36 (0.75–2.46)	0.306		
GLUT1 high, *vs*. low	1.43 (0.72–2.82)	0.307		
PKM2 high, *vs*. low	2.04 (1.06–3.92)	0.033	2.05 (1.05–4.01)	0.036*
MCT4 high, *vs*. low	2.21 (0.67–7.25)	0.191		

OR, odds ratio; CI, confidence interval; LDHA, lactate dehydrogenase A; GLUT1, glucose transporter 1; PKM2, pyruvate kinase isoform M2; MCT4, monocarboxylate transporter 4.
*P < 0.05.

LDHA were significantly lower with CLT (P = 0.049), suggesting that CLT may contribute to a metabolically reprogrammed and less aggressive tumor microenvironment. However, among patients with recurrence, the presence of CLT was linked to higher IRS of LDHA in peritumoral normal tissues (P = 0.047) (Fig. 6).

Glycolytic enzymes and transporter protein messenger RNA expression according to papillary thyroid carcinoma subtype using The Cancer Genome Atlas thyroid cancer data

In PTC subtypes, significant differences in enzyme expression were observed. PKM2 and LDHA expression were

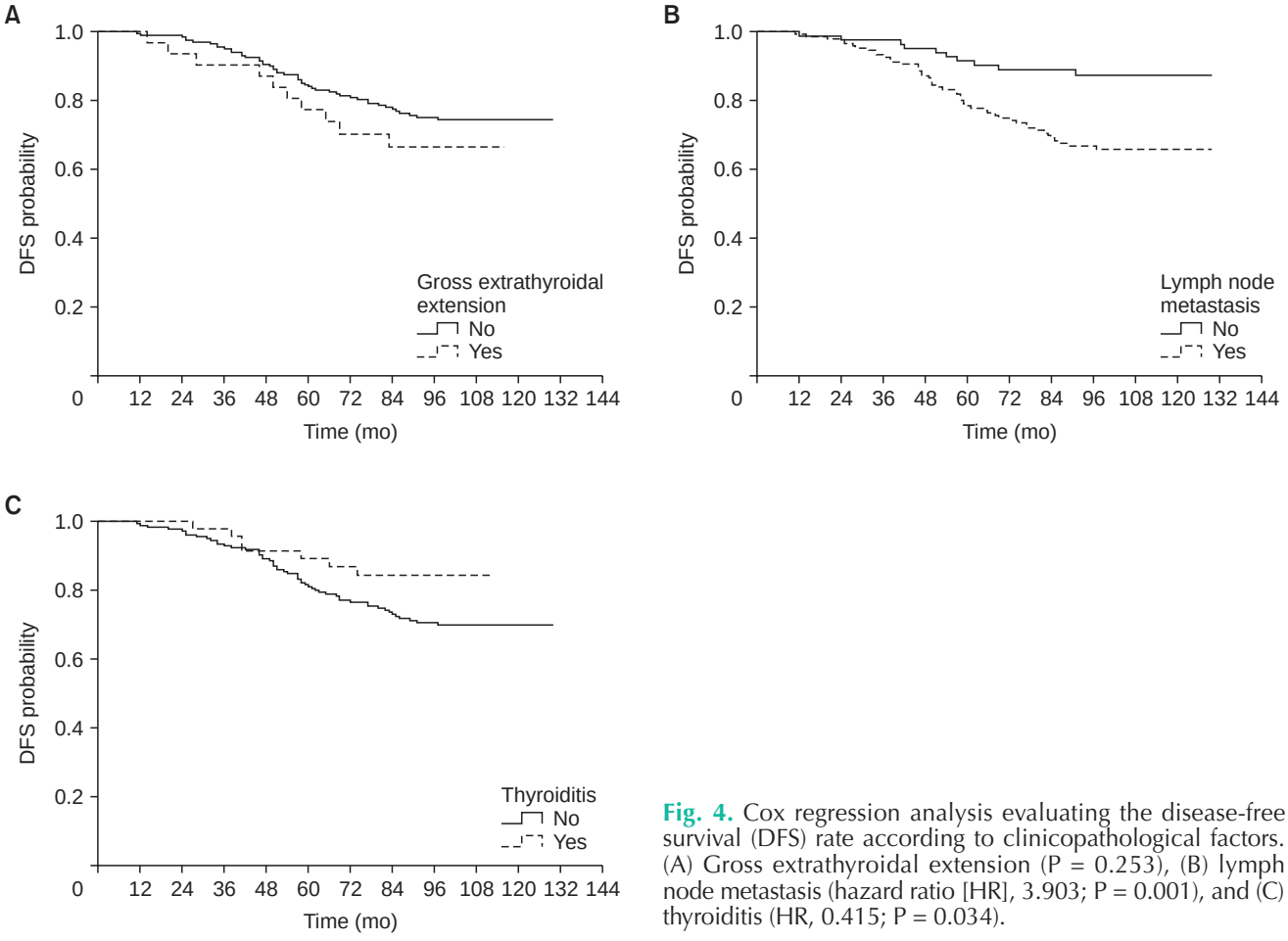

Fig. 4. Cox regression analysis evaluating the disease-free survival (DFS) rate according to clinicopathological factors. (A) Gross extrathyroidal extension (P = 0.253), (B) lymph node metastasis (hazard ratio [HR], 3.903; P = 0.001), and (C) thyroiditis (HR, 0.415; P = 0.034).

highest in the tall cell subtype, followed by the classic, others, and follicular subtypes, with statistically significant differences among the classic, follicular, and tall cell subtypes (P < 0.001). Similarly, for GLUT1 expression, the tall cell and classic subtypes exhibited higher levels than the follicular subtype (P < 0.001). These findings suggest that glycolytic enzyme overexpression is linked to more aggressive PTC subtypes, particularly the tall cell subtype (Fig. 7).

Messenger RNA expression of glycolytic enzymes and transporter proteins according to *BRAF*^{V600E} mutation using The Cancer Genome Atlas thyroid cancer data

mRNA expression levels of LDHA, PKM2, and GLUT1 were significantly elevated in PTC samples harboring the *BRAF*^{V600E} mutation compared to wild-type cases (P = 0.001 for all). High expression of LDHA, PKM2, and GLUT1 was markedly more frequent in the *BRAF*^{V600E} mutation group (34.1%, 34.6%, and 36.9%, respectively) than in the wild-type group (16.5%, 17.3%, and 14.5%). Conversely, low expression levels were predominant in wild-type tumors. These findings suggest a strong association between *BRAF*^{V600E} mutation and glycolytic pathway activation in PTC, underscoring the role of metabolic reprogramming in tumor progression (Table 7).

Univariate and multivariate analysis of risk factors for lymph node metastasis using The Cancer Genome Atlas thyroid cancer data

Univariate analysis of TCGA data indicated that younger age (<55 years), male sex, multifocality, larger tumor size, ETE, advanced T stage, the *BRAF*^{V600E} mutation, and elevated mRNA expression of LDHA, PKM2, and GLUT1 were significantly associated with LNM. In multivariate analysis, younger age, multifocality, advanced T stage (T3/T4), and higher PKM2 mRNA expression remained significant. Younger patients had more than twice the risk of LNM compared to older patients (OR 2.42, P = 0.001), and PKM2 emerged as the strongest predictor (OR 6.91, P = 0.001). Although LDHA and GLUT1 were significant in univariate analysis, they did not retain significance when adjusted for other factors, suggesting that their influence is not independent (Table 8).

These data confirm that while LDHA and GLUT1 may have some impact, PKM2 remains the most critical independent predictor of LNM in PTC patients.

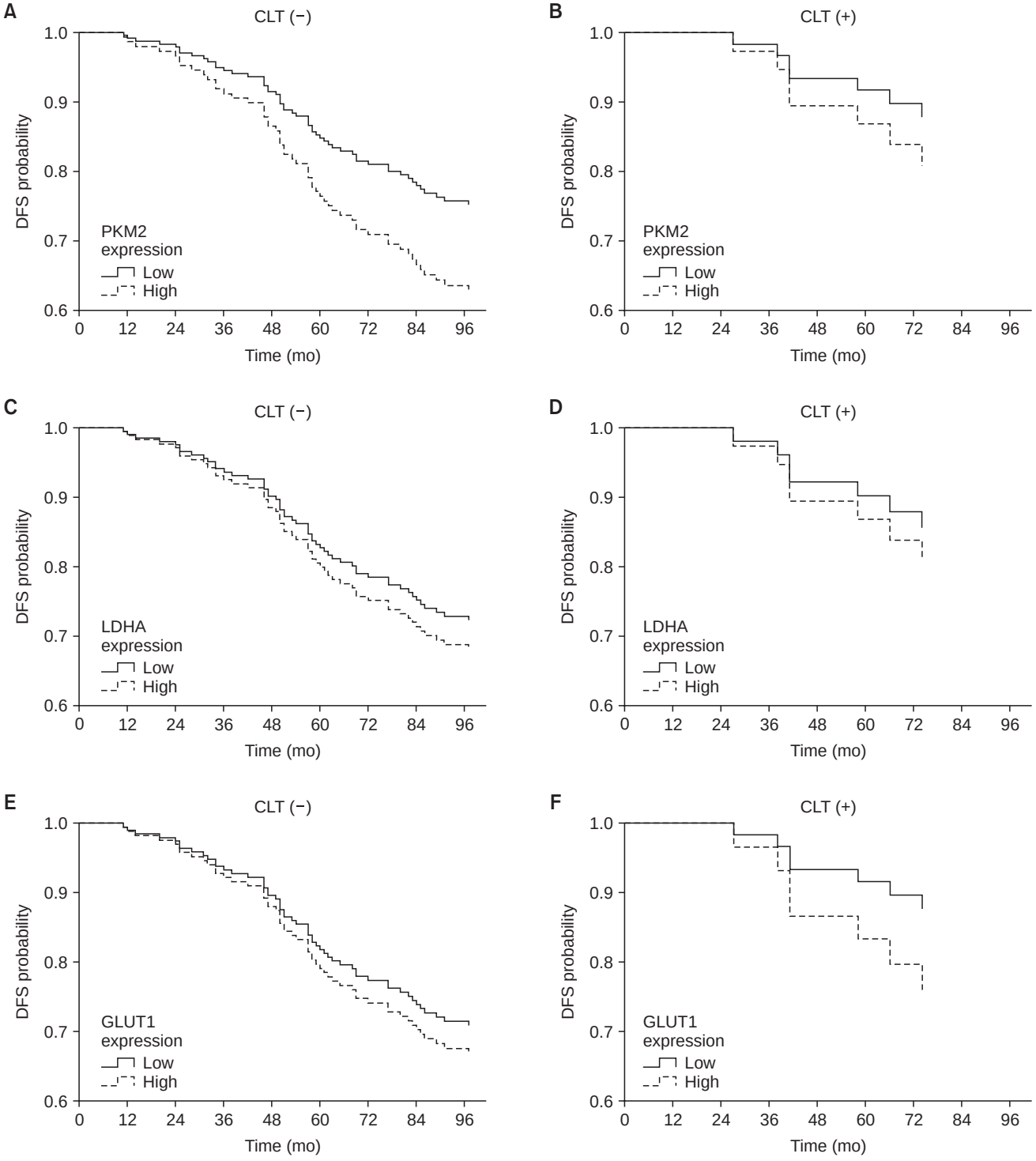

Fig. 5. Cox regression analysis of disease-free survival (DFS) rate based on glycolytic enzyme expression stratified by presence of chronic lymphocytic thyroiditis (CLT). (A) Pyruvate kinase isoform M2 (PKM2) expression without CLT (hazard ratio [HR], 1.76; 95% confidence interval [CI], 1.01–3.06; P = 0.046). (B) PKM2 expression with CLT (HR, 0.20; 95% CI, 0.02–2.31; P = 0.197). (C) Lactate dehydrogenase A (LDHA) expression without CLT (HR, 1.18; 95% CI, 0.69–2.03; P = 0.544). (D) LDHA expression with CLT (HR, 1.37; 95% CI, 0.27–7.07; P = 0.708). (E) Glucose transporter 1 (GLUT1) expression without CLT (HR, 1.17; 95% CI, 0.63–2.18; P = 0.627). (F) GLUT1 expression with CLT (HR, 2.08; 95% CI, 0.47–9.31; P = 0.337).

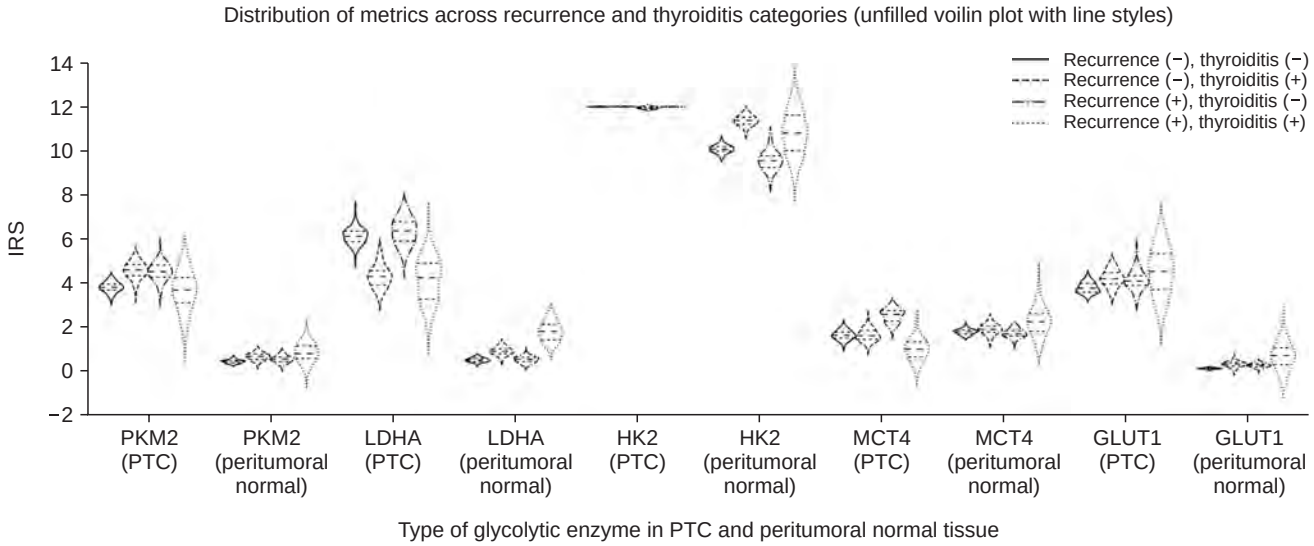

Fig. 6. Immunoreactive scores (IRS) of glycolytic enzymes in peritumoral normal and cancerous tissues accompanied by chronic lymphocytic thyroiditis in the recurrence group. PKM2, pyruvate kinase isoform M2; PTC, papillary thyroid carcinoma; LDHA, lactate dehydrogenase A; HK2, hexokinase 2; MCT4, monocarboxylate transporter 4; GLUT1, glucose transporter 1.

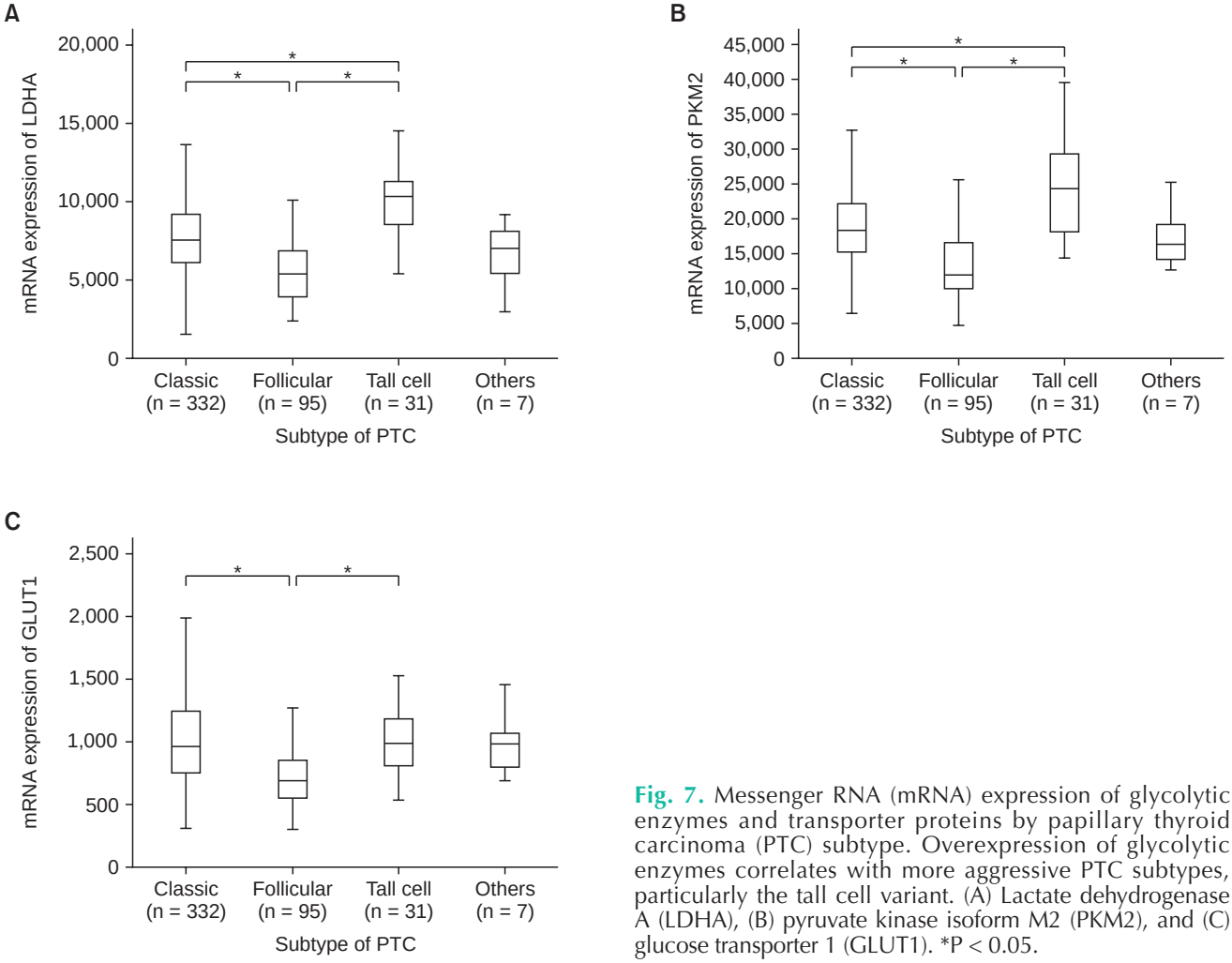

Fig. 7. Messenger RNA (mRNA) expression of glycolytic enzymes and transporter proteins by papillary thyroid carcinoma (PTC) subtype. Overexpression of glycolytic enzymes correlates with more aggressive PTC subtypes, particularly the tall cell variant. (A) Lactate dehydrogenase A (LDHA), (B) pyruvate kinase isoform M2 (PKM2), and (C) glucose transporter 1 (GLUT1). *$P < 0.05$.

Table 7. Messenger RNA (mRNA) expression levels of glycolytic enzymes and transporter proteins according to the presence of *BRAF*^{V600E} mutation

Variable	BRAF wild type (n = 248)	*BRAF*^{V600E} mutation (n = 217)	P-value
LDHA mRNA expression			0.001*
Low	99 (39.9)	19 (8.8)	
Intermediate	108 (43.5)	124 (57.1)	
High	41 (16.5)	74 (34.1)	
PKM2 mRNA expression			0.001*
Low	102 (41.1)	13 (6.0)	
Intermediate	103 (41.5)	129 (59.4)	
High	43 (17.3)	75 (34.6)	
GLUT1 mRNA expression			0.001*
Low	101 (40.7)	19 (8.8)	
Intermediate	111 (44.8)	118 (54.4)	
High	36 (14.5)	80 (36.9)	

Values are presented as number (%).
LDHA, lactate dehydrogenase A; PKM2, pyruvate kinase isoform M2; GLUT1, glucose transporter 1.
*P < 0.05.

DISCUSSION

Extensive research has demonstrated the roles of glycolytic enzymes in various cancers, linking their overexpression to more aggressive behavior and poor outcomes [13,21,23,28-31]. PKM2 and LDHA, for example, are frequently elevated in breast, lung, and colorectal cancers, and their inhibition is being investigated as a therapeutic strategy to curb tumor growth and metastasis [28,29,32,33]. In this study, we aimed to clarify whether the expression of glycolytic enzymes correlates with tumor aggressiveness—namely LNM and ETE—in the presence or absence of CLT.

Compared with normal cells, cancer cells exhibit increased glucose uptake and lactate production, even under aerobic conditions (the Warburg effect), thereby fueling proliferation and progression [8,21,34,35]. Here, we found marked overexpression of glycolytic enzymes and transporters in thyroid cancer tissues, reflecting a metabolic shift favoring enhanced glycolysis.

Table 8. Univariate and multivariate analysis of lymph node metastasis in patients with papillary thyroid carcinoma in TCGA thyroid cancer data

Variable	Univariate		Multivariate	
	OR (95% CI)	P-value	OR (95% CI)	P-value
Age <55 yr, *vs.* ≥55 yr	1.55 (1.04–2.31)	0.031	2.42 (1.48–3.94)	0.001
Male sex	2.37 (1.27–4.44)	0.007	1.42 (0.87–2.33)	0.163
Multifocality	1.46 (1.01–2.12	0.046	1.62 (1.09–2.42)	0.017
Tumor size (cm)	1.20 (1.06–1.35)	0.003	1.07 (0.91–1.26)	0.442
Extrathyroidal extension				
Negative	1		1	
Minimal	2.60 (1.69–3.99)	0.001	1.51 (0.74–3.08)	0.256
Gross	11.95 (2.67–53.51)	0.001	0.94 (0.27–3.29)	0.921
T stage[a]				
T1	1		1	
T2	1.6 (0.98–2.61)	0.06	1.64 (0.96–2.82)	0.070
T3	2.93 (1.80–4.77)	0.001	3.16 (1.83–5.46)	0.001
T4	12.27 (3.38–44.46)	0.001	30.44 (6.07–152.74)	0.001
BRAF^{V600E} mutation	1.47 (1.02–2.12)	0.041	0.89 (0.55–1.44)	0.647
LDHA mRNA expression				
Low	1		1	
Moderate	2.30 (1.42–3.72)	0.001	1.51 (0.82–2.78)	0.184
High	4.03 (2.32–7.00)	0.001	1.82 (0.85–3.87)	0.121
PKM2 mRNA expression				
Low	1		1	
Moderate	3.09 (1.86–5.13)	0.001	3.45 (1.88–6.31)	0.001
High	5.36 (3.02–9.50)	0.001	6.91 (3.37–14.17)	0.001
GLUT1 mRNA expression				
Low	1		1	
Moderate	2.22 (1.40–3.54)	0.001	1.24 (0.70–2.19)	0.466
High	2.17 (1.27–3.69)	0.004	0.69 (0.35–1.37)	0.292

TCGA, The Cancer Genome Atlas; OR, odds ratio; CI, confidence interval; LDHA, lactate dehydrogenase A; mRNA, messenger RNA; GLUT1, glucose transporter 1; PKM2, pyruvate kinase isoform M2; MCT4, monocarboxylate transporter 4.
[a]According to American Joint Committee on Cancer 7th edition.

Metabolic reprogramming in thyroid cancer: increased glycolysis and glutaminolysis

In thyroid cancer, the heightened glycolytic flux in tumor cells is not matched by a corresponding increase in pyruvate oxidation. Instead, pyruvate is predominantly converted to lactate by LDHA. Lactate and pyruvate are then transported either into mitochondria or out of the cell via MCTs. The secreted lactate is often taken up by adjacent cancer cells, creating a feedforward loop that sustains tumor growth. Under hypoxic conditions, glycolysis enables cancer cells to survive in poorly vascularized areas [20,22,36-38]. This adaptation, however, leads to excessive lactate production and reduced extracellular pH, fostering an invasive microenvironment conducive to tumor cell migration.

Beyond glycolysis, many cancer cells rely heavily on glutamine metabolism to support the TCA cycle, thereby maintaining the biosynthesis of fatty acids and amino acids [20,35]. The interplay between glucose and glutamine metabolism allows tumor cells to thrive under metabolically challenging conditions. PKM2, a key enzyme in glycolysis, orchestrates this process by regulating the conversion of PEP to ATP and pyruvate. Numerous studies have linked PKM2 to increased tumor aggressiveness [13,31,39-41].

Role of chronic lymphocytic thyroiditis and the peritumoral microenvironment in papillary thyroid carcinoma

Prior studies show that elevated glycolysis in CD4+ T cells in CLT is driven by enzymes such as GLUT1, HK2, PKM2, and LDHA. The immune response in CLT, particularly through thyroglobulin-specific CD8+ T cells, promotes cytokine release (e.g., interleukin [IL]-6, IL-1β, IL-8), attracting more lymphocytes to the thyroid gland [27,42,43]. Although the prognostic implications of CLT in PTC have been debated, it is often associated with both favorable and unfavorable outcomes [44-48].

A previous study found that the expression of glycolytic enzymes (GLUT1, HK2, PKM2, LDHA) was significantly elevated in thyroid inflammation compared to normal thyroid tissue. This increase correlated with higher extracellular acidification rates and oxygen consumption rates, suggesting that thyroid inflammation may contribute to shaping the peritumoral microenvironment [27].

In this study, CLT was significantly linked to higher rates of gross ETE, suggesting a more invasive local tumor behavior. However, CLT did not emerge as a risk factor for LNM, and patients with PTC and CLT surprisingly exhibited a lower recurrence rate [44,45,47,48]. Thus, while CLT may enhance local tumor invasion, it appears to limit metastatic potential. Inflammatory changes in the thyroid, driven by CLT, may alter cytokine profiles and metabolic pathways, promoting local invasion while simultaneously restraining distant dissemination.

Our data also indicate that glycolytic enzymes such as LDHA and PKM2 are more strongly expressed in the peritumoral normal thyroid tissue of patients with CLT than in those without thyroiditis. This finding suggests that the inflammatory milieu in CLT may drive metabolic reprogramming. While lactate-enriched environments facilitate local invasion, the chronic inflammatory state may also activate immune responses that constrain metastasis. This intricate interplay likely underlies the paradoxical prognostic effects of CLT, which promotes local invasiveness but reduces recurrence.

The fibrotic and inflammatory changes induced by CLT may also act as physical barriers, hindering the dissemination of tumor cells and thereby lowering recurrence rates [46,49]. Thus, the net effect of CLT on PTC outcomes may depend on a balance between metabolic facilitation of local invasion and immune-mediated constraints on distant spread.

Pyruvate kinase isoform M2 and other glycolytic enzymes in papillary thyroid carcinoma prognosis

The *BRAF*^{V600E} mutation is highly prevalent, but its utility as a prognostic marker for tumor aggressiveness—including gross ETE, LNM, and recurrence—remains limited. A significant upregulation of glycolytic enzyme mRNA expression was observed in tumors harboring the *BRAF*^{V600E} mutation. However, in multivariate analysis, only PKM2—not *BRAF*^{V600E} mutation, LDHA, or GLUT1—emerged as an independent risk factor for LNM. These findings suggest that the overexpression of glycolytic enzymes, particularly PKM2, is more strongly associated with tumor aggressiveness than the presence of a *BRAF*^{V600E} mutation. In line with this, we found that PKM2 overexpression was significantly associated with recurrence in PTC, a finding further supported by TCGA data. This dataset also demonstrated a specific correlation between PKM2 overexpression and LNM, reinforcing its role as a key driver of tumor progression.

Conversely, overexpression of LDHA and GLUT1 was not significantly associated with LNM or recurrence. Regardless of the presence or absence of thyroiditis, the majority of glycolytic enzymes showed no significant differences in their distribution. However, LDHA levels were notably lower in PTC with thyroiditis, and even in recurrent cases, its IRS value remained low when thyroiditis was present. In contrast, PKM2 was found to significantly increase the risk of recurrence, independent of the presence of thyroiditis, suggesting that PKM2 plays a role in recurrence irrespective of inflammatory conditions. Although LDHA is a critical enzyme in glycolysis, PKM2 appears to be the most strongly associated with recurrence in PTC [22].

While thyroid cancer generally carries a favorable prognosis, PKM2 overexpression emerged as a key predictor of recurrence, underscoring the potential importance of incorporating glycolytic enzyme profiles into long-term risk stratification.

Although several studies have investigated glycolytic enzyme expression and its link to thyroid cancer malignancy, few have evaluated its relationship with thyroid inflammation. In this study, we confirmed that glycolytic enzyme expression was elevated in malignant tissue, while LDHA expression was notably reduced in the presence of CLT. In contrast, PKM2 expression remained consistently associated with recurrence in patients without CLT. Interestingly, in cases with CLT, the prognosis tended to be better—even when PKM2 expression levels were comparable.

Unlike studies involving animal models and cell lines, this research utilized pathological tissue slides from actual patients. To further enhance the findings, protein-level analysis was performed using immunohistochemical staining of key glycolytic enzymes, enabling a more comprehensive clinical evaluation of the influence of thyroiditis on tumor aggressiveness.

Limitations

This study had several limitations. First, we did not define correlations between protein and mRNA expression. Since TCGA data reflect mRNA expression levels, whereas our study evaluates protein expression through IHC, inherent differences between the 2 expression patterns should be acknowledged as a limitation. Second, we did not define standardized cutoff values for PKM2, LDHA, and GLUT1 expression, which may affect interpretability. Additionally, TCGA data did not provide information on CLT, precluding analysis of its influence on LNM and ETE in that dataset. Further investigation is warranted to elucidate the role of CLT in shaping the tumor microenvironment and influencing PTC outcomes. Despite these limitations, PKM2 emerged as a promising prognostic marker for PTC recurrence.

In conclusion, overexpression of glycolytic enzymes such as LDHA, PKM2, and GLUT1 is associated with PTC. Interestingly, CLT is associated with greater local invasiveness (gross ETE) yet paradoxically lower recurrence. LDHA expression was lower in the presence of CLT, whereas PKM2 was consistently associated with increased recurrence, regardless of inflammatory status. Notably, patients with CLT demonstrated a better prognosis, even when PKM2 expression levels were high, suggesting a potential protective effect of CLT on disease outcomes. Among the examined enzymes, PKM2 may serve as a valuable biomarker for identifying patients at higher risk of recurrence.

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Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Writing – Original Draft: WWK
Writing – Review & Editing: All authors

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