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ORIGINAL ARTICLE – ENDOCRINE TUMORS

Tumor-Infiltrating Lymphocytes as Mediators of the Obesity and Papillary Thyroid Carcinoma Lymph Node Metastasis Association: An Observational Retrospective Cohort Study

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ABSTRACT

Background. Obesity increases the risk of papillary thyroid carcinoma (PTC) and lymph node metastasis (LNM), possibly via modulation of the tumor immunological microenvironment.

Materials and Methods. The STROCSS guideline was followed to conduct a retrospective cohort study. Binary logistic regression analysis with odds ratios (OR) was performed to assess the association between tumor-infiltrating lymphocytes (TILs), obesity, and LNM. Using The Cancer Genome Atlas (TCGA) data, we examined the relationship between immune cell subsets and obesity-regulating molecules in thyroid cancer tissues. The Cox regression risk model was used to analyze the prognosis of thyroid cancer.

Results. After adjusting for confounding factors, our findings revealed that overweight and obesity were associated with a decrease in TIL infiltration (OR 0.876, $p = 0.005$ and OR 0.795, $p = 0.001$, respectively). Furthermore, these conditions were observed to be correlated with increased likelihood of LNM (OR 1.134, $p = 0.005$ and OR 1.307, $p < 0.001$, respectively). On the contrary, TIL infiltration was inversely associated with LNM (OR 0.868, $p < 0.001$). When controlling for TIL infiltration as the sole variable, the combination of obesity and TIL infiltration did not independently predict LNM (adjusted OR 1.442, $p = 0.113$). However, obesity alone was found to elevate the likelihood of LNM (adjusted OR 1.539, $p = 0.02$). Additionally, adiponectin (a crucial adipokine) was reduced in obesity and demonstrated a negative correlation with the abundance of infiltrated dendritic cells and regulatory T cells, as evidenced by TCGA data analysis. Furthermore, ADIPOR2 expression negatively correlated with LNM and positively associated with unfavorable prognosis in PTC, with a hazard ratio of 0.480 ($p = 0.007$).

Conclusions. TIL infiltration may affect obesity-associated PTC LNM. Obesity may affect LNM and result in poor prognosis through ADIPOR2 regulation of antitumor immune cells.

Keywords Tumor-infiltrating lymphocytes · Papillary thyroid cancer · Lymph node metastasis · Obesity · ADIPOR2 · Tumor immunity

The incidence of thyroid cancer is increasing rapidly, and its prevalence is increasing by approximately 13% annually.[1] The predominant pathological type of thyroid cancer, accounting for 85–90% of cases, is papillary. Although most cases of papillary thyroid carcinoma (PTC) have excellent long-term prognoses, highly invasive PTC can develop with rapid lymph node metastasis (LNM) or recurrence. Given the lack of effective treatment, this invasive form of the disease dramatically reduces patients' quality of life and survival.[2] It also increases surgical difficulty and risk. LNM is

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an early, and the most common, clinical feature of PTC, with a probability of 23–77%.[3] Thus, a thorough investigation of the mechanism of LNM in PTC is of great importance for diagnosis and prognosis prediction.

In parallel with thyroid cancer, the incidence of obesity, a significant health and social problem, is increasing rapidly. Obesity is a well-documented risk factor for the development of 13 malignancies, including thyroid cancer.[4–7] However, whether obesity-induced systemic metabolic changes affect immune cells in the local tumor microenvironment remains unclear. Thus, basic and clinical research on the association between obesity and thyroid cancer is needed.[8–11]

In clinical practice, we have noted that the pathological features of PTC are more aggressive in obese patients, and we have conducted a series of studies. In previous studies, we found that obesity is a risk factor for aggressive PTC growth and LNM.[12,13] The tumor immunological microenvironment (TIME), consisting of immune cells and their effector molecules, plays a key role in LNM.[14] Tumor-infiltrating immune cells involved in LNM have antitumor (e.g., effector T cells (CD4+/CD8+), natural killer cells, dendritic cells (DCs), macrophages, and neutrophils) and pro-tumor (e. g., regulatory T cells (Tregs) and myeloid-derived suppressor cells) functions, and the ratio thereof determines the "fate" of the tumor.[15,16] When tumor-promoting immune cells predominate, tumor cells evade immune surveillance, and metastasis occurs. Furthermore, the entry of tumor-infiltrating lymphocytes (TILs; e.g., T and natural killer cells) into tumors from the bloodstream alters the TIME and may influence LNM.[17,18] Wang et al.[19] observed that obesity can lead to the dysfunction and depletion of antitumor immune cells in the TIME in mice, primates, and obese patients. Thus, obesity may promote LNM through modulation of TIL infiltration and TIME (i.e., tumor-infiltrating immune cell ratio) alteration.[15,16] However, previous studies have focused primarily on tumor cells and their regulatory axis in colon and endometrial cancers,[11,20,21] and the mechanisms by which TIME alteration influences tumor growth remains unclear. In this study, our primary aim is to unravel the intricate interplay between obesity, TILs, and PTC with LNM. Specifically, we seek to determine the role of TILs in mediating the relationship between obesity and PTC–LNM, with a keen focus on elucidating the underlying mechanisms that

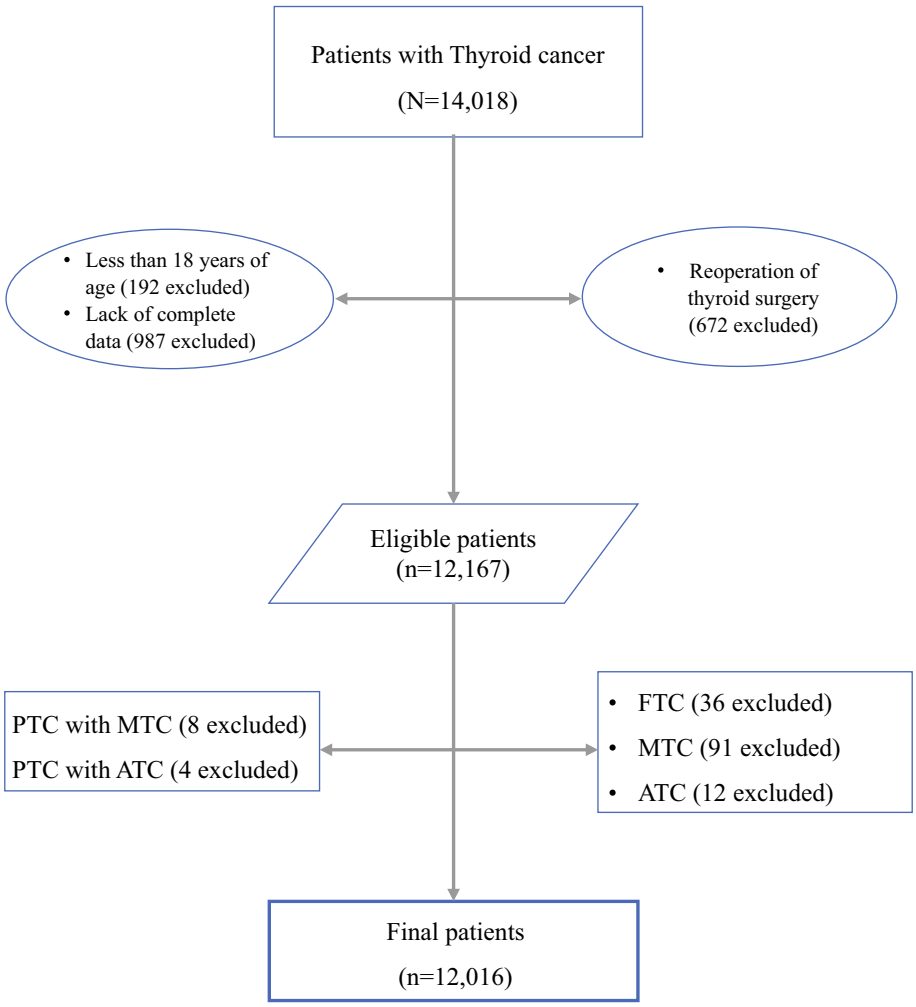


FIG. 1 Flow of study inclusion

TABLE 1 Basic information

Characteristic	Total (n = 12,016) Mean ± SD or N (%)	Characteristic	Total (n = 12,016) Mean ± SD or n (%)
Age	42.92 ± 9.41	Lymph node metastasis	
Height (cm)	164.13 ± 6.92	N0	7047 (58.6%)
Weight (kg)	65.70 ± 12.17	N1	4969 (41.4%)
BMI (kg/m^2)	24.28 ± 3.48	Number of lymph node metastases	1.75 ± 3.64
BMI group[1]		Tumor stage[2]	
Underweight	389 (3.2%)	T1a	9224 (76.5%)
Normal	5615 (46.7%)	T1b	2077 (17.3%)
Overweight	4350 (36.2%)	T2	432 (3.6%)
Obesity	1662 (13.8%)	T3a	18 (0.1%)
Thyroid Function		T3b	213 (1.8%)
TSH (mIU/L)	3.13 ± 2.95	T4a	52 (0.4%)
FT3 (pmol/L)	4.53 ± 1.02	High T stage	316 (2.6%)
FT4 (pmol/L)	15.90 ± 6.09	Clinical stage[2]	
TgAb (ng/mL)	64.07 ± 276.14	I	11549 (96.0%)
TPOAb (IU/mL)	47.37 ± 103.26	II	455 (3.8%)
Pathological features		III	7 (0.1%)
Tumor infiltrating lymphocytes	5082 (42.3%)	IVA	–
Tumor diameter > 10 mm	2730 (22.7%)	IVB	5 (0.1%)
Multifocality	4858 (40.4%)	Distant metastasis	19 (0.2%)
Extrathyroidal extension	3109 (25.9%)		

BMI body mass index, *TSH* thyroid stimulating hormone, *FT3* free triiodothyronine, *FT4* free thyroxine

[1]According to the CN-BMI definition

[2]Determined by the 8th edition AJCC/UICC TNM Staging System

*$p < 0.05$

**$p < 0.01$

involve modulation of the immune microenvironment and the pivotal role of obesity-regulating molecules.

MATERIALS AND METHODS

Ethical Approval

Ethical approval for this study (Ethical Committee no. 2019040806) was provided by the Health Care Ethics Committee of the China–Japan Union Hospital of Jilin University, Changchun, China on 8 April 2019. All patients have signed informed consent forms before surgeries. The patients have obtained written informed consent to agree to publish this case report and the attached pictures. The editor-in-chief of this journal can provide copies of written consent forms for review upon request.

Study Population

This retrospective study was conducted using data from patients who underwent radical PTC treatment at the China–Japan Union Hospital, Jilin University, between 2009 and 2021. This study was conducted according to the principles set forth in the Declaration of Helsinki. The inclusion criteria were as follows: age ≥ 18 years, PTC diagnosis confirmed by postoperative histopathological examination, no other history of cancer, and complete medical information. Patients who underwent secondary surgery and those with PTC combined with other pathological subtypes (follicular, medullary, or anaplastic) were excluded. This study was reported in line with the STROCSS criteria.[22]

Standard Operation

A consistent surgical approach was adopted for all patients, beginning with the complete resection of the affected thyroid lobe where the lesion was located. Subsequently, as the second step, prophylactic central neck dissection (CND) was performed for all patients with thyroid cancer. The CND was performed in a caudal-to-cranial direction. The surgical technique for prophylactic nodal dissection included ipsilateral CND. Depending on the surgical strategy, the surgeon may alternate between the thyroidectomy and CND components of the operation.

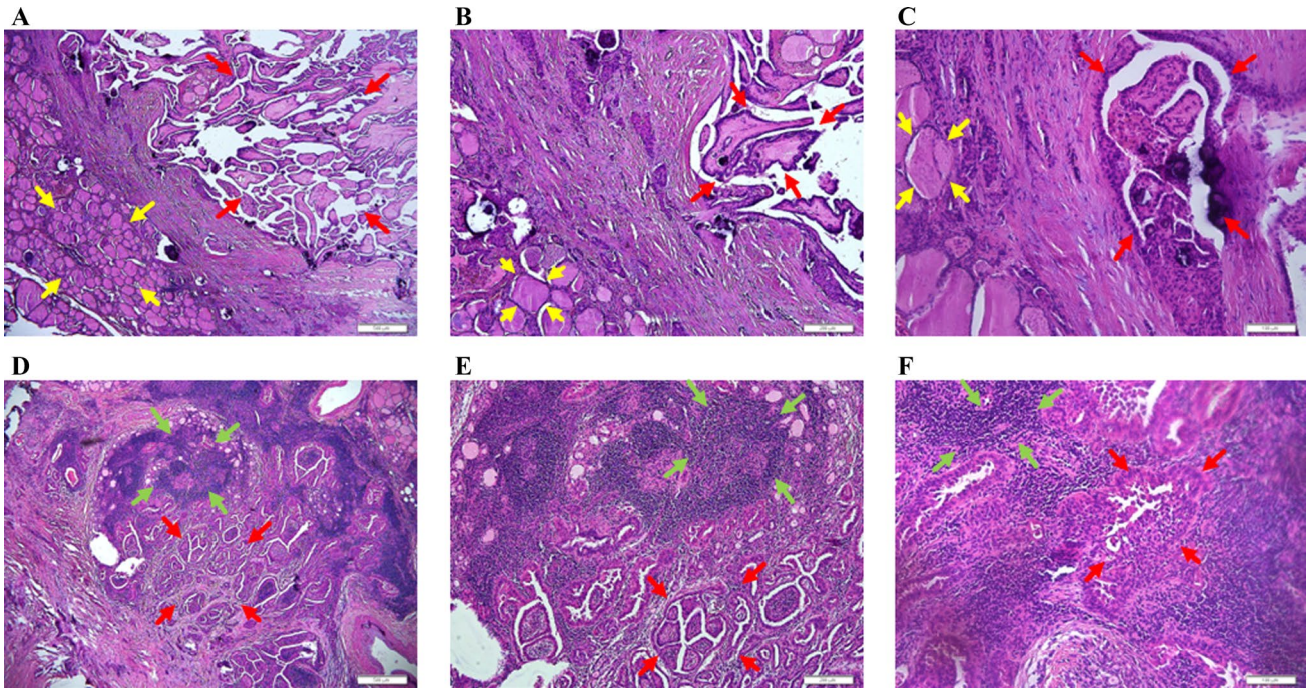


FIG. 2 HE-stained lymphocytic infiltrates in PTC tissues. Yellow arrows, thyroid follicular cells; red arrows, thyroid cancer cells; green arrows, lymphocyte infiltration

Model	OR (95% CI)	*P* value
Model 1		
CN-BMI		<0.001**
<18.5	1.307 (1.064-1.605)	0.011*
18.5-23.9	Reference	
24-27.9	0.834 (0.769-0.903)	<0.001**
≥28	0.673 (0.600-0.753)	<0.001**
Model 2		
CN-BMI		<0.001**
<18.5	1.218 (0.958-1.549)	0.108
18.5-23.9	Reference	
24-27.9	0.876 (0.799-0.962)	0.005**
≥28	0.795 (0.698-0.906)	0.001**
Model 3	0.951 (0.941-0.961)	<0.001**
Model 4	0.966 (0.954-0.978)	<0.001**

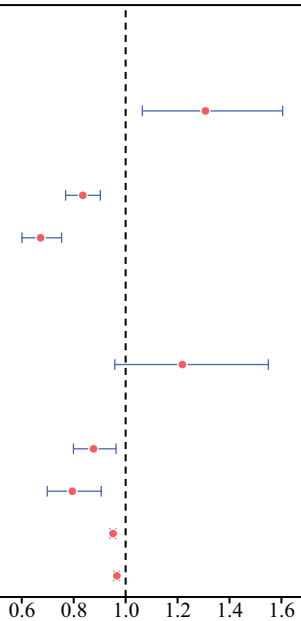


FIG. 3 Conditional logistic regression analysis of BMI and risk for TILs. *BMI* body mass index, *OR* odds ratio, *CI* confidence interval.Logistic regression model 1 included CN -BMI quartiles as covariates. Model 2 included CN-BMI quartiles, lgTSH, lgFT3, lgFT4, lgTgAb, lgTPOAb, and high T stage as covariates. Model 3 included BMI as covariates. Model 4 included BMI, lgTSH, lgFT3, lgFT4, lgTgAb, lgTPOAb, and high T as covariates.*P<0.05, **P<0.01

Study Variables

Data on patients' general characteristics (age, sex, height, weight, body mass index (BMI), thyroid function (thyroid-stimulating hormone, TSH), free triiodothyronine (FT3), free thyroxin (FT4), thyroid peroxidase antibody (TPO-Ab), and thyroglobulin antibody (Tg-Ab) levels) and tumor features (maximum diameter, multifocality, extrathyroidal extension (ETE), LNM, presence of TILs, TNM stage, and clinical stage) were collected from the hospital's electronic medical records and entered into a Microsoft Office Access (Microsoft Corporation, Redmond, WA, USA). According

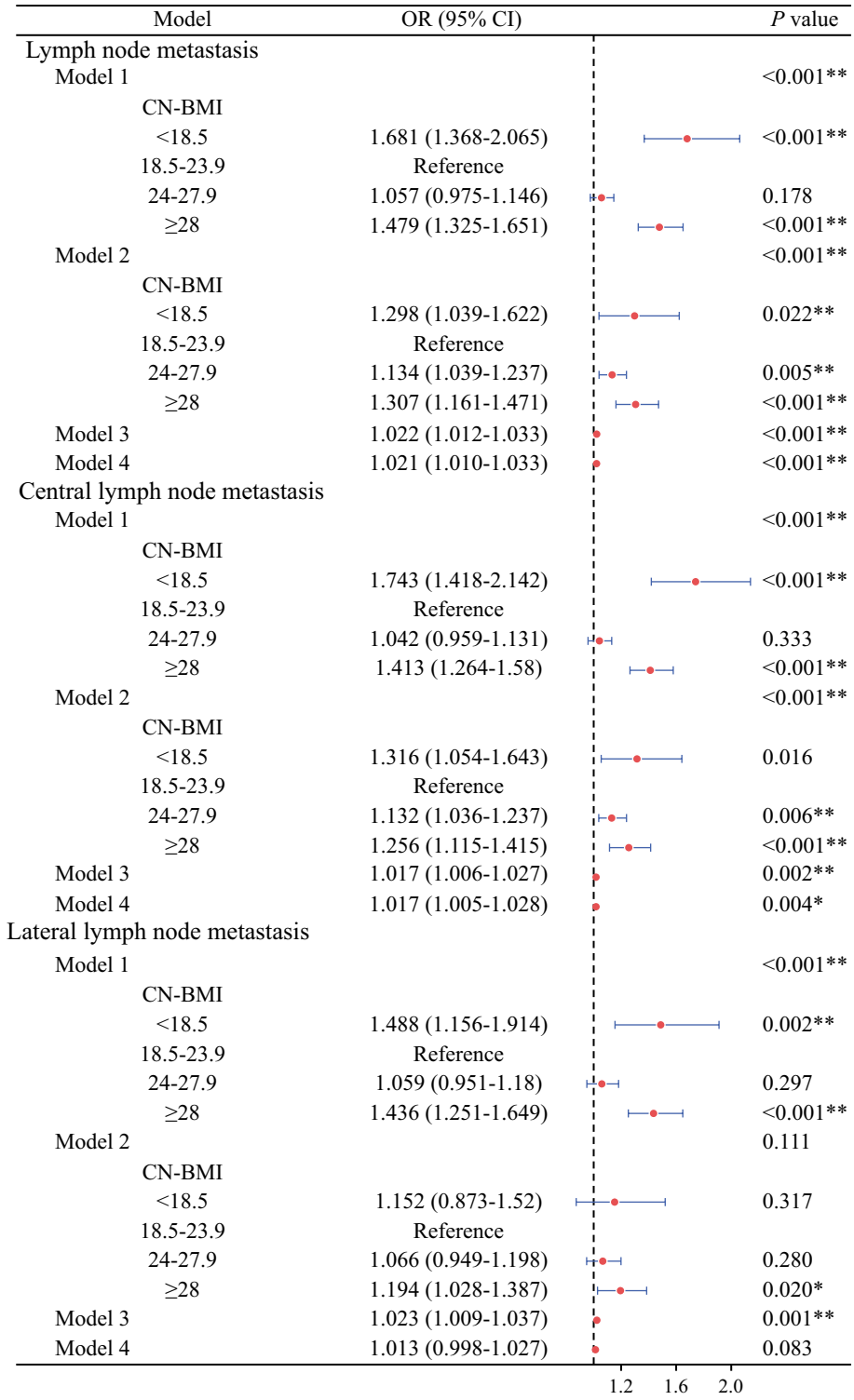


FIG. 4 Conditional logistic regression analysis of BMI and risk for lymph node metastasis. *BMI* body mass index, *OR* odds ratio, *CI* confidence interval.Logistic regression model 1 included CN -BMI quartiles as covariates.Model 2 included CN-BMI quartiles, lgTSH, lgFT4, TILs, tumor diameter >10 mm, extrathyroidal extension, and high T stage as covariates. Model 3 included BMI as covariates. Model 4 included BMI, lgTSH, lgFT4, TILs, tumor diameter >10 mm, extrathyroidal extension, and high T stage as covariates.*P<0.05, **P<0.01

to the Chinese classification, patients were characterized as underweight (BMI < 18.5 kg/m^2), normal weight (BMI 18.5–23.9 kg/m^2), overweight (BMI 24–27.9 kg/m^2), or obese (BMI ≥ 28 kg/m^2).[23] Maximum tumor diameter > 1 cm, ETE, LNM, high T stage (T3/T4), and high clinical stage (III/IV) were regarded as aggressive pathological features. Histopathological findings were confirmed by the pathologists. Moderate to high risk of recurrence was defined by the presence of any of the following criteria: more than five lymph node metastases, metastatic lymph nodes with a maximum diameter exceeding 0.2 cm, minimal ETE (T3) involving only the periprostatic fascial planes

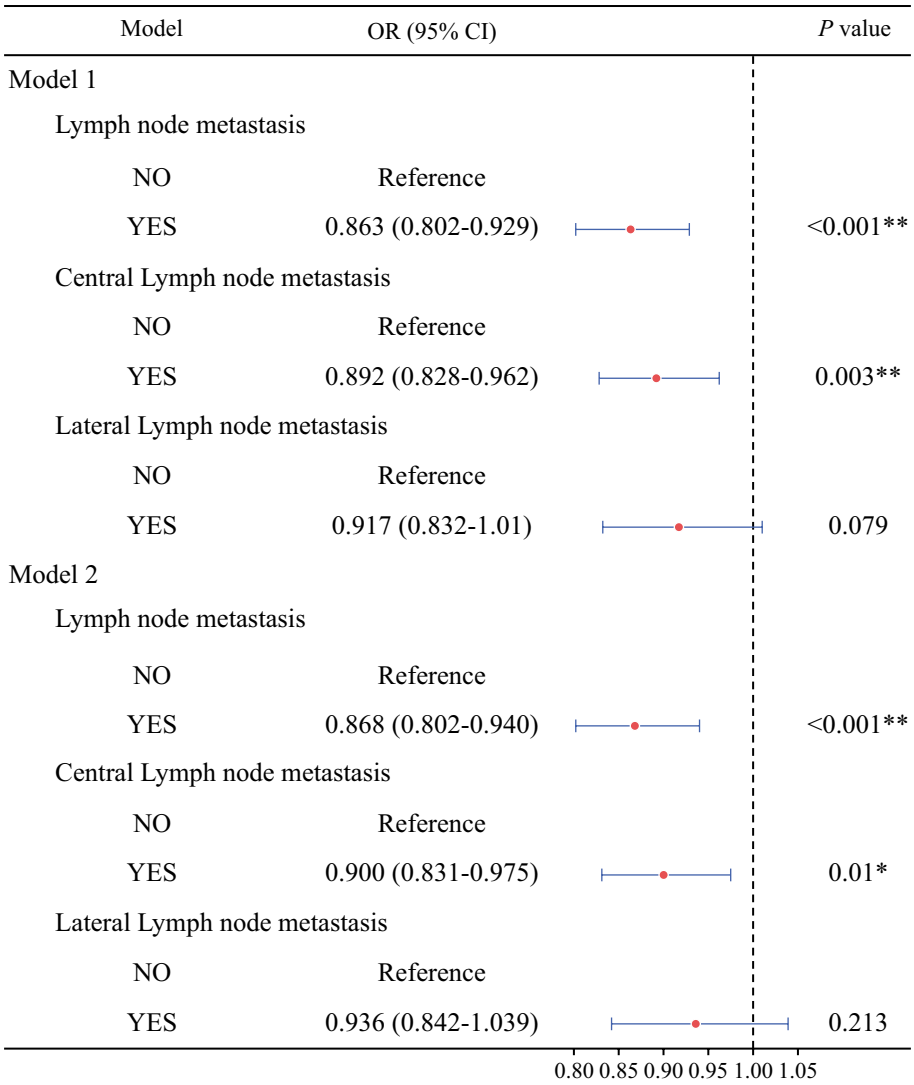


FIG. 5 Conditional logistic regression analysis of TILs and risk for lymph node metastasis. *BMI* body mass index, *OR* odds ratio, *CI* Logistic regression model 1 included TILs quartiles as covariates.Model 2 included age, BMI, lgTSH, lgFT4, TILs, tumor diameter >10 mm, extrathyroidal extension, and high T stage as covariates.*P<0.05, **P<0.01

or gross ETE (T4) invading surrounding organs, aggressive histological subtypes or vascular invasion, macroscopically visible residual tumor tissue, or distant metastasis (M1).

Bioinformatics Analyses

We collected RNA sequencing data for thyroid cancer from The Cancer Genome Atlas (TCGA) public database. We examined the expression of the Acrp30 receptors AdipoR1, AdipoR2, and T-cadherin (CDH13), and the leptin receptor (LEPR) in thyroid cancer tissues. We further analyzed the relationship between ADIPOR2 and LEPR expression, and the degree of immune infiltration in thyroid cancer tissues. Statistical analyses and visualization of these data were performed using R (version 4.2.1; R Foundation for Statistical Computing, Vienna, Austria; https://www.R-project.org/.12c) and the ggplot2 package (Springer–Verlag New York, 2016) for the latter. Transcripts per million were used for data normalization. The proportional hazards assumption test was performed, and Cox regression analysis was conducted using the survival package. Variables that met the set p-value threshold on univariate analysis were included in the multivariate Cox model for modeling.

Statistical Analysis

The statistical analyses for this study were rigorously conducted using SPSS software (version 22.0; IBM Corporation, Armonk, NY, USA). The methodology chosen was comprehensive and specifically tailored to the characteristics

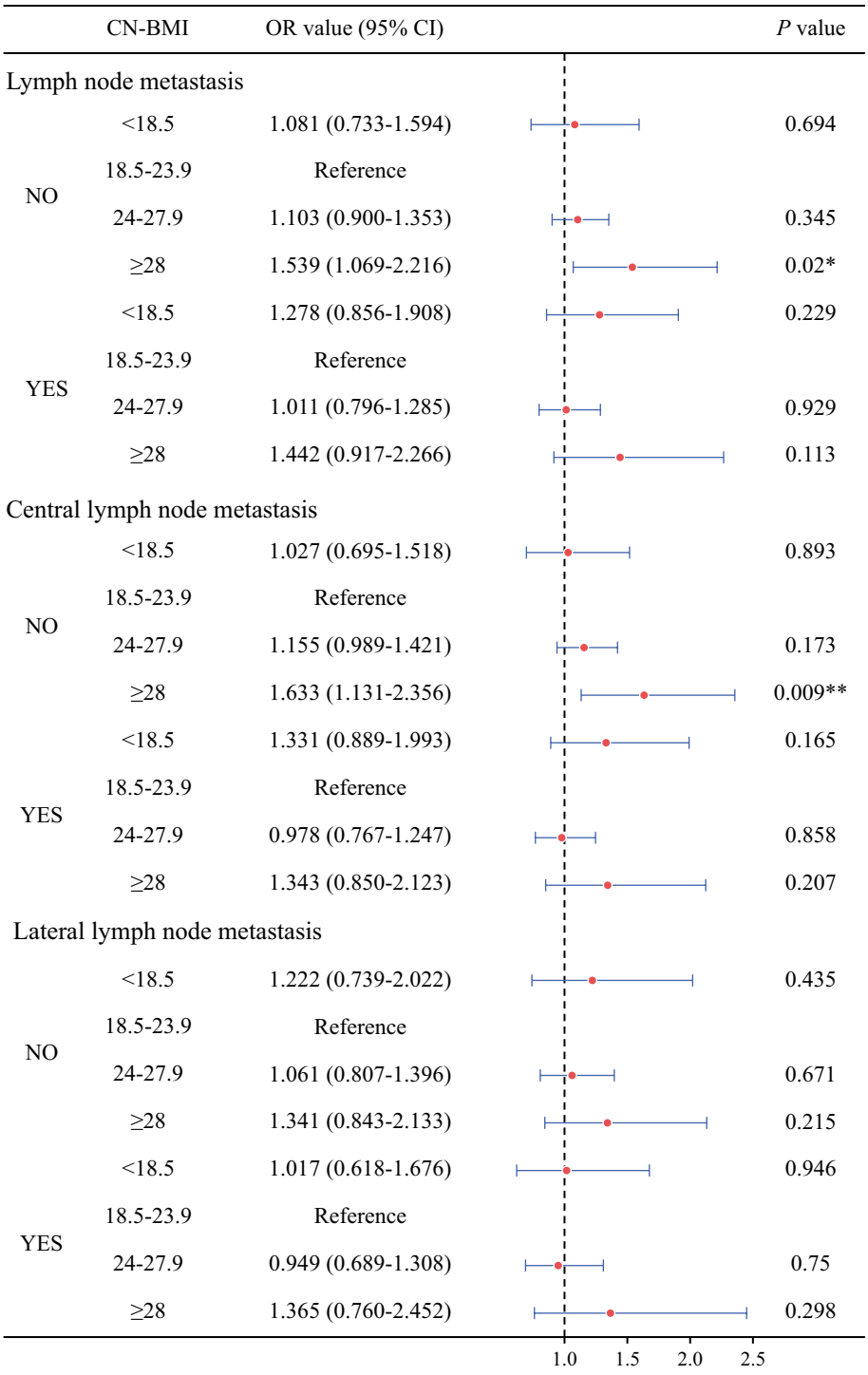


FIG. 6 Conditional logistic regression analysis of TILs and risk for lymph node metastasis in different BMI group. *BMI* body mass index, *OR* odds ratio, *CI* Logistic regression model 1 included TILs quartiles as covariates. Model 2 included age, BMI, lgTSH, lgFT4, TILs, tumor diameter >10 mm, extrathyroidal extension, and high T stage as covariates. **P*<0.05, ***P*<0.01

of the variables being examined. For continuous variables, including biochemical and clinical measurements, descriptive statistics are presented as mean ± standard deviation, providing a robust overview of their distribution patterns. Categorical variables, denoting the presence or absence of specific conditions or characteristics, were reported as counts and frequencies, facilitating an understanding of proportions and occurrences. To assess statistical significance between groups for continuous variables that adhered to a normal distribution, Student's *t*-test was applied for pairwise comparisons, while the analysis of variance (ANOVA) was employed for multiple group comparisons. These tests enabled us to evaluate the statistical differences in means. For categorical variables, the chi-squared (χ^2) test served as the primary tool for examining associations between groups. In instances where the expected cell counts were

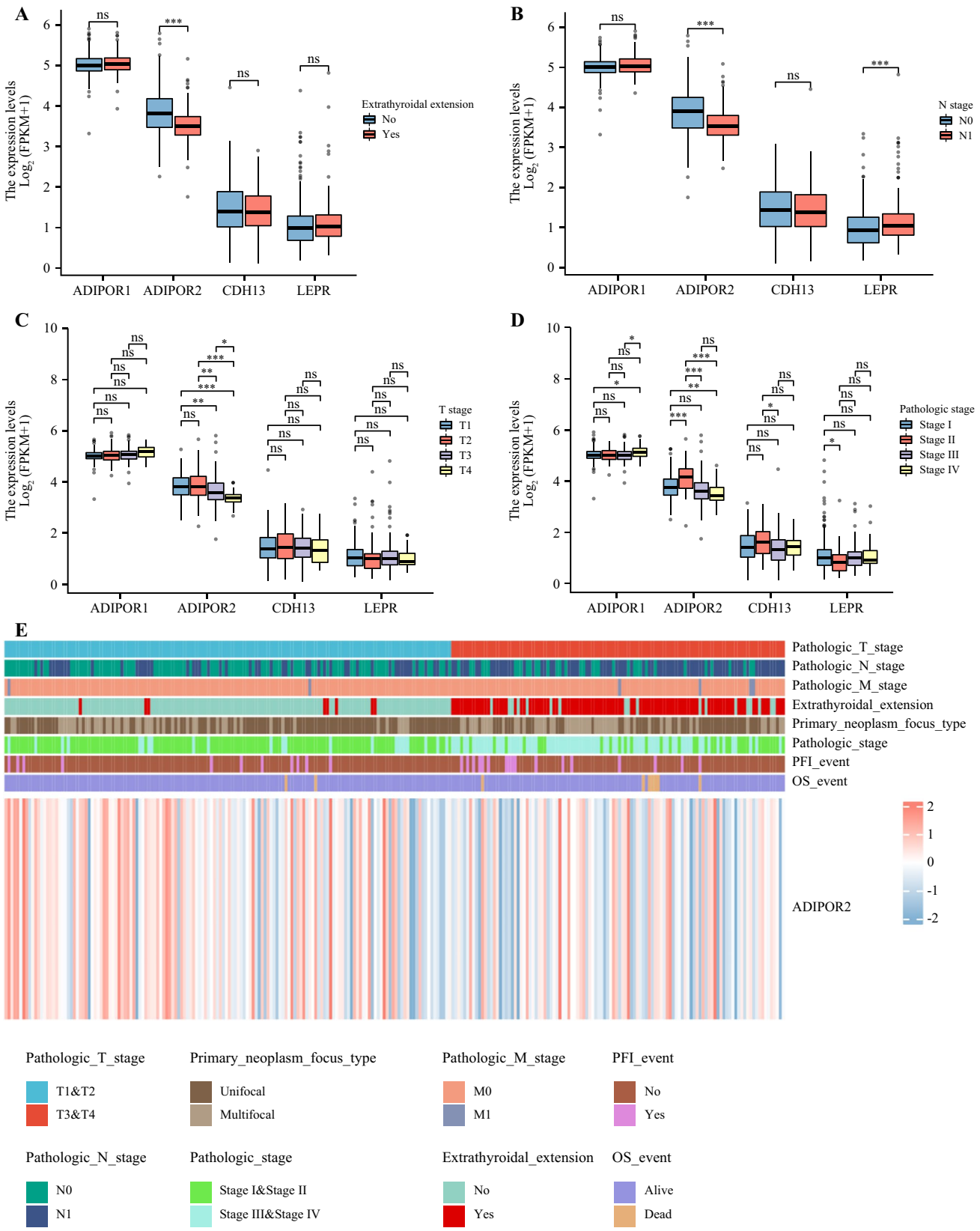


FIG. 7 Relationships between the expression of adiponectin and leptin receptors, and pathological features of PTC. ETE (**A**), N stage (**B**), T stage (**C**), and pathological stage (**D**) and heatmap of ADIPOR2 gene expression and clinical variables (**E**)

deemed too small for the χ^2 test to be valid, Fisher's exact test was utilized to maintain the statistical rigor. To quantify the linear relationship between TIL infiltration and various demographic and clinical variables, bivariate Pearson's correlation coefficients were computed. This analysis shed light on potential confounding or mediating factors influencing the study outcomes.

Furthermore, to evaluate the intricate relationships between obesity, TIL infiltration, and LNM, binary logistic regression models were formulated. These models generated odds ratios (ORs) and their corresponding 95% confidence intervals (CIs), enabling us to assess the strength and direction of these associations. Additionally, to estimate the risk of progression-free intervals, Cox proportional hazards regression models were employed. Hazard ratios (HRs) and 95% CIs were derived from these models, allowing for the quantification of the effects of various factors, notably TIL infiltration and obesity, on disease progression. For variables that did not conform to a normal distribution, such as TSH, FT3, FT4, TPO-Ab, and Tg-Ab, logarithmic transformation was applied to normalize their distributions, thereby enabling the application of parametric statistical tests. Throughout the analysis, a significance level of $p < 0.05$ was set, indicating that observed differences were deemed statistically significant if the probability of their occurrence due to chance alone was less than 5%.

RESULTS

Clinical Features of Patients with PTC

The sample comprised 12,016 patients with PTC (male:female ratio, 1:3.7) with a mean age of 42.92 ± 9.41 years (Fig. 1). Patient characteristics are summarized in Table 1. According to BMI, 36.2% of the patients were overweight and 13.8% were obese. The mean TSH, FT3, FT4, TPO-Ab, and Tg-Ab values were 3.13 ± 2.95 mIU/L, 4.53 ± 1.02 pmol/L, 15.91 ± 6.09 pmol/L, 47.37 ± 103.26 IU/mL, and 19.08 ± 43.43 ng/mL, respectively. Tumors had maximum diameters > 1 cm in 22.7% of the cases and were multifocal in 40.4% of the cases. ETE, LNM, high T stage, and distant metastasis were found in 25.9%, 41.4%, 2.6%, and 0.2% of the cases, respectively. TILs were present in 42.3% of the patients (Fig. 2).

Obesity and TIL Infiltration

Univariate analysis revealed that higher BMI, as well as elevated FT3 and FT4 levels, and advanced T stage were associated with decreased TIL infiltration, while increased TSH, TPO-Ab, and Tg-Ab levels were associated with increased risk of decreased TIL infiltration (Supplementary Fig. 1). After accounting for potential confounders, our analysis identified overweight and obesity as independent factors that were significantly associated with decreased TIL infiltration (OR 0.876 (95% CI 0.799–0.962), $p = 0.005$ and OR 0.795 (95% CI 0.698–0.906), $p = 0.001$, respectively; Fig. 3).

Obesity and LNM

On binary logistic regression analysis, adjusted for multiple factors including age, BMI, TSH and TPO-Ab levels, TILs, maximum tumor diameter greater than 1 cm, ETE, and high T stage (Supplementary Fig. 2), being overweight was found to be associated with a modest increase in the odds of LNM (OR 1.134 (95% CI 1.039–1.237), $P = 0.005$), while obesity exhibited a stronger association with increased odds of LNM (OR 1.307 (95% CI 1.161–1.471), $p < 0.001$).

Further analysis showed that obesity increased the risk of LNM in the central and lateral neck regions (adjusted OR 1.256 (95% CI 1.115–1.415), $p < 0.001$ and adjusted OR 1.194 (95% CI 1.028–1.387), $p = 0.02$, respectively; Fig. 4). The total number of LNMs and the number of LNMs in the central and lateral neck regions correlated linearly and positively with the BMI (Supplementary Fig. 3).

TIL Infiltration and LNM

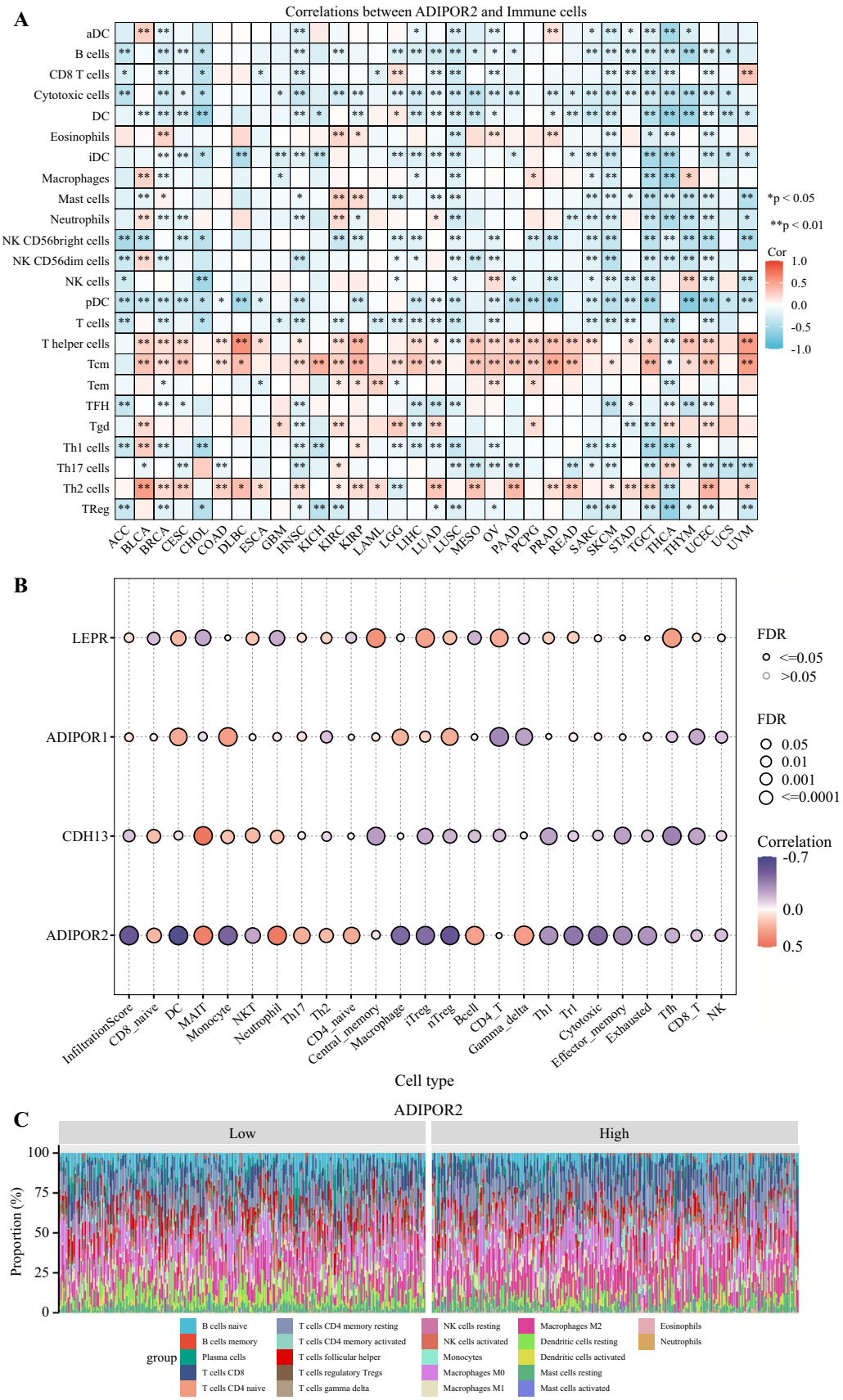
Binary logistic regression analysis, adjusted for confounders, revealed a significant association between increased TIL infiltration and a reduced likelihood of lymph node metastasis (LNM) (OR 0.868, 95% CI 0.802–0.940, $p < 0.001$). Further analysis clarified that this association was specific to central LNM (adjusted OR 0.900 (95% CI 0.831–0.975), $p = 0.01$; Fig. 5).

TIL Infiltration Affects the Association between Obesity and LNM

Obesity was observed to be associated with an elevated likelihood of LNM (adjusted OR 1.539 (95% CI 1.069–2.216), $p = 0.02$), whereas the combination of obesity and TIL infiltration did not emerge as an independent indicator of heightened LNM likelihood (adjusted OR 1.442 (95% CI 0.917–2.266), $p = 0.113$). A congruent pattern was discernible for LNM in the central region (Fig. 6).

TIL Infiltration and Tumor Recurrence

The binary logistic regression analysis revealed a significant correlation between decreased TIL infiltration and medium/high risk of recurrence in PTC (OR 0.857 (0.785–0.935), $p < 0.001$). As indicated in Supplementary


A
Correlations between ADIPOR2 and Immune cells
aDC
B cells
CD8 T cells
Cytotoxic cells
DC
Eosinophils
iDC
Macrophages
Mast cells
Neutrophils
NK CD56bright cells
NK CD56dim cells
NK cells
pDC
T cells
T helper cells
Tcm
Tem
TFH
Tgd
Th1 cells
Th17 cells
Th2 cells
TReg
ACC BLCA BRCA CESC CHOL COAD DLBC ESCA GBM HNSC KICH KIRC KIRP LAML LGG LIHC LUAD LUSC MESO OV PAAD PCPG PRAD READ SARC SKCM STAD TGCT THCA THYM UCEC UCS UVM
*p < 0.05
**p < 0.01
Cor
1.0
0.5
0.0
-0.5
-1.0
B
LEPR
ADIPOR1
CDH13
ADIPOR2
InfiltrationScore CD8_naive DC MAIT Monocyte NKT Neutrophil Th17 Th2 CD4_naive Central_memory Macrophage iTreg nTreg Bcell CD4_T Gamma_delta Th1 Tr1 Cytotoxic Effector_memory Exhausted Tfh CD8_T NK
Cell type
FDR
<=0.05
>0.05
FDR
0.05
0.01
0.001
<=0.0001
Correlation
-0.7
0.0
0.5
C
ADIPOR2
Low
High
Proportion (%)
100
75
50
25
0
group
B cells naive
B cells memory
Plasma cells
T cells CD8
T cells CD4 naive
T cells CD4 memory resting
T cells CD4 memory activated
T cells follicular helper
T cells regulatory Tregs
T cells gamma delta
NK cells resting
NK cells activated
Monocytes
Macrophages M0
Macrophages M1
Macrophages M2
Dendritic cells resting
Dendritic cells activated
Mast cells resting
Mast cells activated
Eosinophils
Neutrophils

◂**FIG. 8** The relationship between ADIPOR2 and TIL infiltrated immune cells. **A** Infiltration of tumor immune cell subsets in pan-cancer; **B**, **C** the relationship between the infiltration degree of immune cell subsets and the expression of adiponectin receptors and leptin receptor in thyroid cancer

Table 1, tumor recurrence risk is multifaceted and influenced by numerous factors. Upon further adjustment for these confounding variables, no significant association was found between the degree of TIL infiltration and medium/high risk of recurrence (adjusted OR 0.905 (0.765–1.069), $p = 0.241$) (Supplementary Table 2).

LNM and Poor Prognosis Associated with Key TIL Molecules

Bioinformatic analysis was conducted using TCGA data from 460 patients with thyroid cancer (229 with LNM). Adiponectin and leptin are the main adipokines secreted by adipocytes. Through TCGA data, ADIPOR2 was found to be significantly downregulated and LEPR was found to be significantly upregulated in patients with LNM (Fig. 7A–D). Further analysis of the heatmap of ADIPOR2 gene expression and clinical variables revealed that the expression of ADIPOR2 was significantly negatively correlated with poor prognosis, including high T staging, N1, M1, ETE, progression-free interval (PFI), and overall survival (OS) (Fig. 7E). According to tumor immune infiltration analysis, ADIPOR2 was negatively correlated with the infiltration of most immune cells in pan-cancer (Fig. 8A). The number of antitumor immune cells (DCs and Tregs) correlated negatively with ADIPOR2 and positively with LEPR expression (Fig. 8B,C). The expression levels of CD80, CD86 (DC markers), interleukin-2 receptor alpha (IL2RA), and forkhead box protein 3 (FOXP3; Treg markers) were strongly associated with ETE and LNM (Fig. 9A–D). Through TCGA data, Cox regression revealed a better prognosis for patients with high ADIPOR2 expression (HR 0.480 (95% CI 0.280–0.810), $P = 0.007$) and worse prognosis for those with high FOXP3 expression (HR 2.000 (95% CI 1.050–3.790), $p = 0.035$; Fig. 9E,F). CD80, CD86, and IL2AR expression was not associated with prognosis (Supplementary Fig. 5A–D). ADIPOR2 was significantly negatively correlated with DC markers (CD80 and CD86) and Treg markers (IL2RA and FOXP3) (Fig. 9G, H). The core cell type table displays data for a panel of eight cell types that are found in thyroid tissues (from the Human Protein Atlas (https://www.proteinatlas.org)) (Supplementary Fig. 6).

Otherwise, they bind to adiponectin receptors (ADIPOR1, ADIPOR2, and CDH13) and leptin receptors (LEPR) to play pro-cancer/anti-cancer roles (Supplementary Fig. 4) (from the Human Protein Atlas (https://www.proteinatlas.org)). The immunohistochemical results of ADIPOR2, CDH13, LEPR, CD86, CD80, IL2RA, and FOXP3 (there are no protein data for ADIPOR1 in thyroid tissue in the database) were analyzed by the Human Protein Atlas (https://www.proteinatlas.org). Compared with normal thyroid tissues, ADIPOR2 was significantly downregulated in PTC tissues, while CD80 and CD86 were significantly upregulated (Fig. 10).

Through TCGA data, further analysis of ADIPOR2 as a prognostic indicator of PFI across various cancers revealed that ADIPOR2 was a favorable prognostic marker for thyroid cancer and kidney renal clear cell carcinoma but an unfavorable prognostic marker for adrenocortical cancer, lung adenocarcinoma, and pancreatic adenocarcinoma (Supplementary Fig. 7). In thyroid cancer, ADIPOR2 was unrelated to prognostic indicators, such as overall survival (OS) and disease-specific survival (DSS) (Supplementary Figs. 8, 9). The COX regression risk model was utilized to predict PFI in thyroid cancer, taking into account factors such as T stage, M stage, pathologic stage, age, ETE, and ADIPOR2 as prognostic indicators (Table 2). To accurately assess the prognosis of patients with thyroid cancer, a nomogram multifactor regression prediction model was employed, which was based on the significant variables identified in the Cox regression risk model. Figure 11 illustrates the nomogram prediction model, which incorporates the significant predictors identified through Cox regression analysis. By incorporating these factors, the nomogram allows for a comprehensive and individualized assessment of prognosis, taking into account the unique characteristics of each patient.

DISCUSSION

Our analysis revealed that overweight and obesity were associated with decreased TIL infiltration and an increased risk of LNM, while TIL infiltration itself was inversely related to LNM. Notably, the combination of obesity and TIL infiltration did not independently confer an increased risk for LNM. These findings suggest that obesity may contribute to an elevated risk of LNM by diminishing TIL infiltration. Additionally, we observed a positive correlation between ADIPOR2 and antitumor immune cells, and a negative correlation with both LNM and poor prognosis.

TILs and Tumor

Malignant tumor development induces the production of a large number of lymphocytes, and lymphocyte infiltration is common in breast,[24] gastric,[25] and colon[26] cancers. The presence of TILs in tumors is associated with improved clinical outcomes. TILs may also influence tumor invasion and metastasis.[27,28] However, TILs are strongly influenced by breast cancer subtypes. Hormone receptor-negative breast

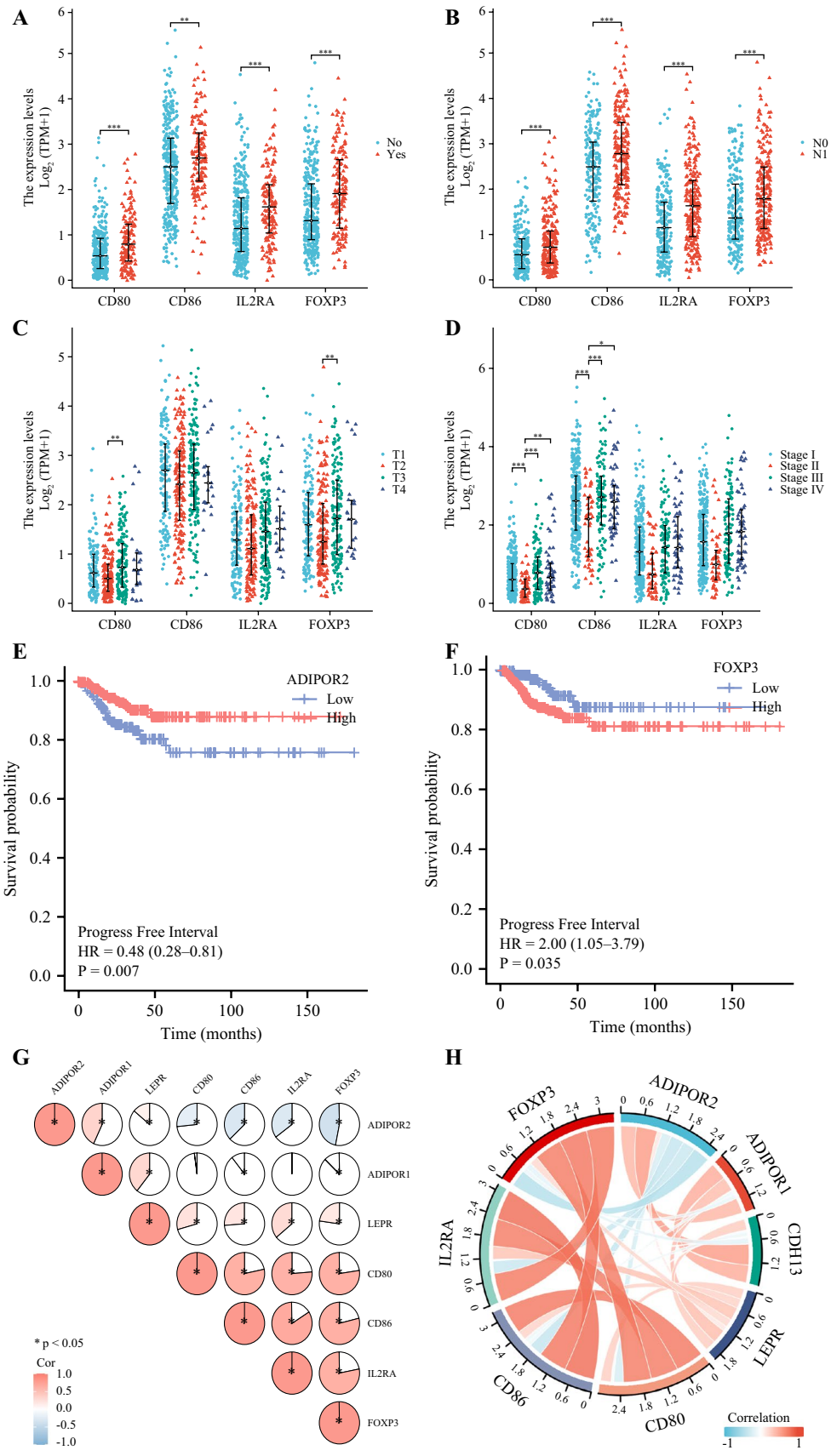


FIG. 9 The relationship between clinical features of thyroid cancer and DC and Treg markers. ETE (**A**), N stage (**B**), T stage (**C**), pathalogic slage (**D**), relationships of ADIPOR2 (**E**) and FOXP3 (**F**) expression levels to PTC prognosis; pie chart (**G**) and chord diagram (**H**) of the correlation between adiponectin and leptin receptors and DC and Treg markers

cancers, such as human epidermal growth factor receptor 2-enriched and triple-negative breast cancers, are associated with greater TIL density than hormone receptor-positive breast cancers.[29,30] Hormone receptor-negative breast cancers, notably human epidermal growth factor receptor 2 (HER2)-enriched and triple-negative breast cancers, exhibit a higher density of TILs compared with hormone receptor-positive breast cancers. This observation underscores the need for tailored therapeutic approaches that leverage the unique immune landscapes of different breast cancer subtypes to enhance antitumor immunity and improve patient outcomes.

Regarding our findings, the binary logistic regression analysis initially indicated a statistically significant correlation between decreased TIL infiltration and a medium/high risk of recurrence in PTC (OR 0.857 (0.785–0.935), $p < 0.001$). Upon meticulous adjustment for these confounding variables, our subsequent analysis failed to uncover a significant direct association between the degree of TIL infiltration and the medium/high risk of recurrence, with an adjusted odds ratio of 0.905 (0.765–1.069) and a *p*-Value of 0.241. This suggests that while TIL infiltration may play a role in modulating the tumor microenvironment, its predictive value for recurrence risk in PTC may be obscured by the complexity of other influencing factors. Further research is warranted to clarify the intricate interplay between TILs, tumor characteristics, and the host immune response in thyroid cancer, which could potentially lead to the development of more accurate prognostic models and targeted therapeutic strategies.

TILs and LNM

In our analysis, we unexpectedly encountered a disparity in the influence of TILs on central versus lateral neck lymph node metastasis, despite the fact that lateral neck LN metastasis is a more pivotal prognostic indicator in thyroid cancer. This observation underscores the intricate interplay between immune responses and tumor biology, which can vary significantly across anatomical sites.

We posit that the difference in TIL activity may stem from distinct microenvironments in the central and lateral neck compartments. The lateral neck, being more distal to the primary tumor site, may encounter a weaker initial immune response, allowing for the development of a more immunosuppressive milieu that favors LN metastasis. Alternatively, distinct immune cell subsets or signaling pathways may be at play in these regions, leading to varied TIL efficacy.

TILs and Obesity

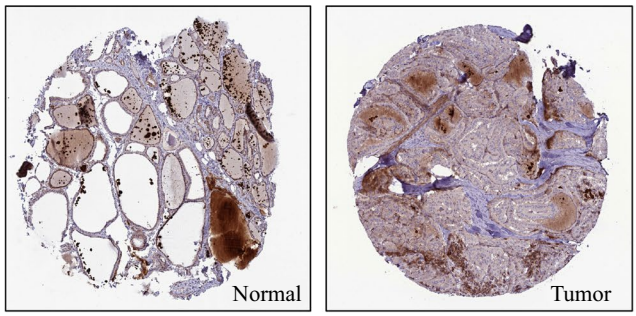
Multiple links between obesity and cancer risk have been identified, including increased levels of growth hormone, leptin, insulin, and adipokines, which can directly promote tumor transformation and progression.[31,32] Recent studies have suggested that obesity, through its complex metabolic derangements, creates an immunosuppressive milieu that indirectly fosters tumorigenesis.[33] In addition, Wang et al.[19] hypothesized that leptin, a hormone abundantly secreted by adipose tissue in obesity, triggers a cascade of events leading to programmed cell death protein 1 (PD-1) upregulation in T cells, thereby diminishing their antitumor efficacy.

High-fat diets induce obesity, impair the infiltration and function of CD8+ T cells in the tumor microenvironment in mice, and promote tumor growth. The abundance of natural killer and CD8+ T cells was greatly reduced in the tumors of obese mice. Residual CD8+ TILs are functionally and metabolically impaired, and thus unable to control tumor growth, in the context of obesity.[34] CD8+ T cell infiltration of endometrial tumors was found to correlate negatively with the BMI, and weight loss was associated with increased programmed death ligand 1 expression and numbers of infiltrating T cells in tumors.[35] Thus, obesity is associated with a "cold" tumor phenotype in humans and mice. In patients with endometrial cancer, weight loss results in tumor regression and restoration of immune cell infiltration. These findings highlight the suppressive effect of obesity on CD8+ T cell antitumor immunity, which can be partially reversed by weight loss and/or immunotherapy.[36]

TIL and Adipokine

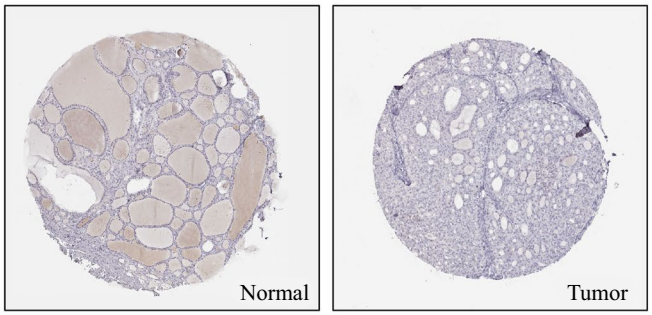
T cell depletion and dysfunction are major factors in obesity-associated LNM of tumors. Leptin, a crucial adipokine secreted by adipose tissue, has emerged as a key player in modulating the immune landscape within the tumor microenvironment. Its ability to induce programmed cell death protein 1 upregulation not only promotes T cell depletion but also contributes to a more immunosuppressive milieu in melanoma, offering fresh insights into the complex interplay between obesity and immune dysfunction.[37] Dyck et al.[36] recently demonstrated that T cell depletion and dysfunction provide novel links between obesity and the increased risk of endometrial cancer. In a mouse model, obesity reduced the number of CD8+ T cells in tumors and their antitumor activity, thereby accelerating tumor growth.[36] In the present study, the investigation delves deeper into the mechanisms underpinning obesity's impact on the immune response against cancer. Specifically, obesity was found to hinder the infiltration of tumor-infiltrating lymphocytes (TILs), a vital component of the antitumor immune response. Additionally, ADIPOR2, a receptor intimately linked to obesity, was shown to negatively regulate the number of antitumor immune cells, such as dendritic cells (DCs) and regulatory T cells (Tregs), further exacerbating the immunosuppressive state. Surprisingly, however, patients exhibiting high

ADIPOR2



Quantity: 75%-25% Quantity: 25%

LEPR



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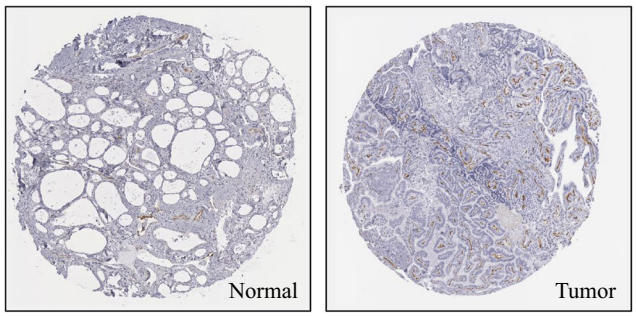
CDH13



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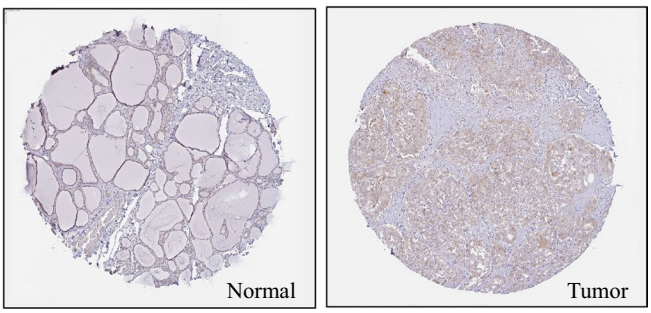
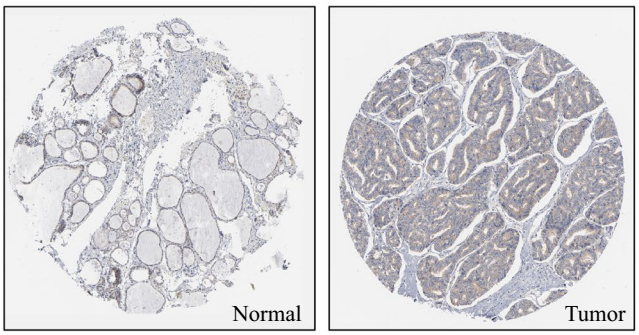
CD80



Quantity: 25% Quantity: 75%-25%

CD86



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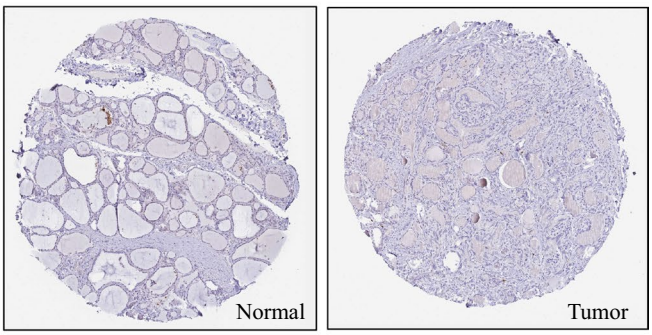
IL2RA



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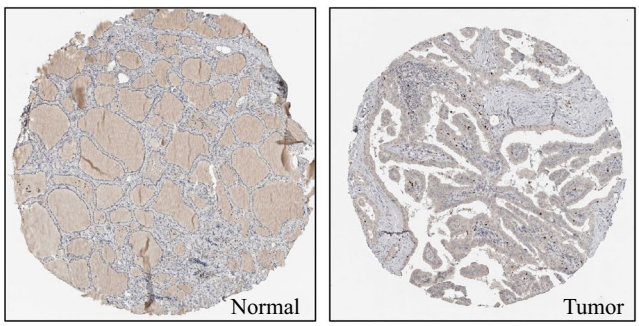
FOXP3



Quantity: None Quantity: None

◂**FIG. 10** Immunohistochemical analysis of the differences in adipokine receptors and key immune cell proteins between normal thyroid tissues and tumor tissues. [1]Immunohistochemical images were retrieved from the Human Protein Atlas database (https://www.proteinatlas.org), a comprehensive resource for exploring the human proteome across tissues and organs. [Uhlén M, Fagerberg L, Hallström BM, et al. Proteomics. Tissue-based map of the human proteome. *Science*. 2015;347(6220):1260419. https://doi.org/10.1126/science.1260419; Uhlen M, Zhang C, Lee S, et al. A pathology atlas of the human cancer transcriptome. *Science*. 2017;357(6352):eaan2507. https://doi.org/10.1126/science.aan2507]

levels of ADIPOR2 expression paradoxically exhibited better prognosis, suggesting that the role of ADIPOR2 in cancer outcomes may be more nuanced and context-dependent than initially thought. This finding underscores the need for further research to fully elucidate the complex mechanisms governing the interplay between adipokines, obesity, and the immune system in cancer progression and prognosis.

TILs and Tumor Microenvironment

The tumor microenvironment contains cancer-associated fibroblasts, angiogenic vascular cells, and infiltrating immune cells, which play important roles in tumor invasion and metastasis.[27,28] Among these cells, peritumoral lymphocytes (TILs) serve as simple indicators of tumor-associated immune responses. T cells and other lymphocytes play roles in external immune defense, internal monitoring, and stability. When normal cells become tumor cells, the immune system mobilizes these cells to deeply penetrate the tumor tissue and recognize and kill tumor cells. For example, CD8+ T cells can differentiate into cytotoxic T cells that recognize and bind to T-cell receptors.[38] Upon transformation of normal cells into tumor cells, the immune system mounts a robust response, mobilizing TILs to deeply infiltrate tumor tissues and execute their antitumor functions. For instance, CD8+ T cells, a key subset of TILs, differentiate into cytotoxic T cells that recognize and bind to specific tumor antigens presented by T-cell receptors, thereby facilitating tumor cell recognition and elimination.

TILs and Subset

In the intricate landscape of the immune microenvironment of thyroid cancer, the subpopulations of TILs, particularly CD8+, CD4+, and regulatory T cells (Tregs), occupy pivotal positions. These lymphocyte subsets not only mirror the immunological status of the tumor but also directly engage in either inhibiting or promoting tumor growth.

CD8+ T cells, functioning as cytotoxic T lymphocytes (CTLs), assume a primary antitumor role in thyroid cancer. They recognize and directly eliminate cancer cells presenting tumor-associated antigens through the release of cytotoxic molecules such as perforin and granzyme, inducing tumor cell apoptosis. The infiltration density and functional status of CD8+ T cells frequently serve as crucial biomarkers for thyroid cancer prognosis. Notably, a high density of CD8+ T cell infiltration is frequently associated with favorable clinical outcomes, underscoring their central role in tumor immune surveillance.[39]

CD4+ T cells, or helper T cells, indirectly exert antitumor effects in the immune response to thyroid cancer by activating other immune cells such as CD8+ T cells, natural killer cells (NKs), and macrophages. They secrete an array of cytokines, including IFN-γ and IL-2, which facilitate the activation and proliferation of immune cells. Studies indicate that substantial variations in circulating immune cells among individuals with differentiated thyroid cancer contribute to differing treatment outcomes. A low absolute CD4+ T cell count has been linked to improved clinical outcomes and a heightened ability for adaptation and recovery.[40]

Tregs (regulatory T cells) represent a distinct T cell population that dampens immune responses. In thyroid cancer, Tregs facilitate tumor immune evasion and proliferation by secreting inhibitory cytokines (e.g., IL-10, TGF-β) and expressing immune checkpoint molecules (e.g., PD-1, CTLA-4), thereby suppressing the activation of effector T cells. Findings have illuminated the significance of CCR8+ Tregs in fostering an immunosuppressive microenvironment in colorectal cancer (CRC) tumors through functional inhibition of CD4+ Tconvs and CD8+ T cells, suggesting the potential application of CCR8-targeted therapy for CRC.[41] Similar mechanisms may also underlie their role in thyroid cancer, emphasizing the need for further investigation.

In light of our current findings, a pivotal future clinical endeavor lies in devising targeted therapeutic strategies capable of reversing the immunosuppressive influence of obesity on TILs, thereby mitigating the risk of LNM and enhancing patient prognosis. This pursuit may encompass the employment of medications that modulate adipokine signaling pathways, for instance, leptin antagonists, or the development of tailored immunotherapies aimed at bolstering TIL functionality specifically in obese cancer patients. Notably, further rigorous clinical trials are imperative to substantiate these approaches and ascertain their safety profiles and therapeutic efficacy across diverse patient demographics.

CONCLUSIONS

The present study had several limitations. The degree of TIL infiltration was not graded, preventing examination of its relationship with LNM and obesity. In addition, because the study was retrospective, we could not identify specific TIL subpopulations. This will be a topic for future research.

TABLE 2 Prediction of progression-free interval using COX regression risk model

Characteristics	Total (*n*)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	*p*-Value	Hazard ratio (95% CI)	*p*-Value
Pathologic T stage	510				
T1	143	Reference		Reference	
T2	169	3.558 (1.197–10.575)	0.022*	2.494 (0.591–10.519)	0.213
T3	175	5.345 (1.865–15.320)	0.002*	2.764 (0.528–14.472)	0.229
T4	23	9.914 (2.897–33.925)	< 0.001**	0.815 (0.082–8.131)	0.861
Pathologic N stage	462				
N0	229	Reference		Reference	
N1	233	1.636 (0.924–2.895)	0.091	0.935 (0.396–2.211)	0.879
Pathologic M stage	295				
M0	286	Reference		Reference	
M1	9	7.305 (2.780–19.197)	< 0.001**	6.116 (1.135–32.946)	0.035*
Pathologic stage	510				
Stage I	288	Reference		Reference	
Stage II	52	1.369 (0.514–3.648)	0.530	1.207 (0.267–5.460)	0.807
Stage III	113	2.203 (1.141–4.254)	0.019*	1.575 (0.384–6.465)	0.528
Stage IV	57	4.062 (2.016–8.185)	< 0.001**	2.184 (0.400–11.912)	0.367
Gender	512				
Female	373	Reference		Reference	
Male	139	1.708 (0.982–2.969)	0.058	1.064 (0.463–2.444)	0.884
Age	512	1.020 (1.003–1.037)	0.018*	1.023 (0.989–1.058)	0.186
Histological type	512			–	–
Classical	366	Reference		–	–
Follicular	101	0.578 (0.245–1.363)	0.210	–	–
Tall cell	36	2.030 (0.908–4.538)	0.085	–	–
Other	9	1.021 (0.140–7.433)	0.984	–	–
Extrathyroidal extension	494			–	–
No	340	Reference		Reference	
Yes	154	1.891 (1.102–3.246)	0.021*	0.975 (0.308–3.083)	0.965
Primary neoplasm focus type	502			–	–
Multifocal	233	Reference		–	–
Unifocal	269	0.966 (0.561–1.663)	0.900	–	–
Neoplasm location	506			–	–
Bilateral	88	Reference		–	–
Isthmus	22	0.363 (0.046–2.869)	0.336	–	–
Left lobe	178	0.979 (0.447–2.144)	0.957	–	–
Right lobe	218	0.904 (0.415–1.966)	0.798	–	–
Thyroid gland disorder history	454			–	–
Normal	286	Reference		–	–
Nodular Hyperplasia	68	0.809 (0.314–2.083)	0.660	–	–
Lymphocytic Thyroiditis	74	0.940 (0.414–2.131)	0.881	–	–
Other, specify	26	1.502 (0.531–4.250)	0.443	–	–
ADIPOR2	512			–	–
Low	256	Reference		Reference	
High	256	0.509 (0.289–0.897)	0.019*	0.710 (0.315–1.604)	0.410
FOXP3	512			–	–
Low	256	Reference		–	–
High	256	1.136 (0.665–1.940)	0.640	–	–
CD80	512			–	–

Table 2 (continued)

Characteristics	Total (*n*)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	*p*-Value	Hazard ratio (95% CI)	*p*-Value
Low	256	Reference		–	–
High	256	1.311 (0.766–2.243)	0.323	–	–
CD86	512			–	–
Low	256	Reference		–	–
High	256	1.237 (0.724–2.112)	0.437	–	–
IL12A	512			–	–
Low	255	Reference		–	–
High	257	0.883 (0.517–1.508)	0.650	–	–
ADIPOR1	512			–	–
Low	256	Reference		–	–
High	256	1.504 (0.874–2.589)	0.141	–	–
LEPR	512			–	–
Low	256	Reference		–	–
High	256	0.833 (0.488–1.422)	0.503	–	–
CDH13	512			–	–
Low	256	Reference		–	–
High	256	0.962 (0.564–1.641)	0.888	–	–

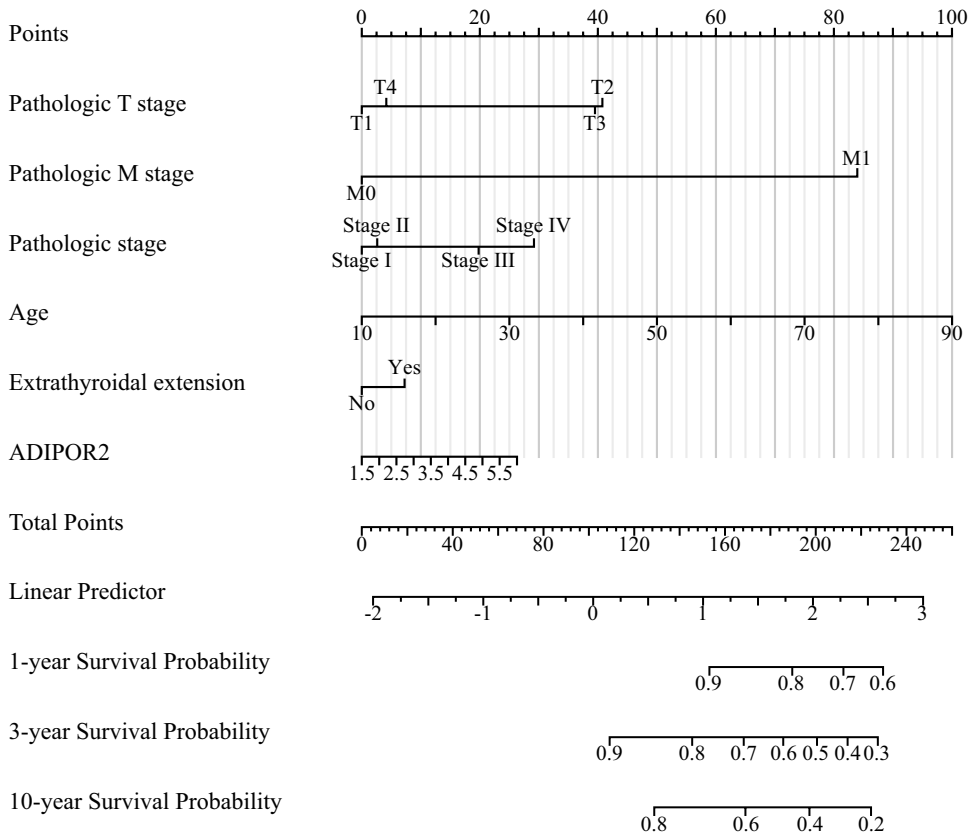


FIG. 11 Nomogram multifactor regression (PFI) prediction model for thyroid cancer

The clinical and bioinformatics findings of this study suggest that TILs are involved in obesity-associated PTC LNM, enriching our understanding of its progression.

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DATA AVAILABILITY This paper is not based on previous communication with society or meetings. The raw data supporting the conclusions of this article will be made available by the authors without any undue reservation.

DISCLOSURES The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

ETHICAL APPROVAL This study was approved by the Health Care Ethics Committee of the China–Japan Union Hospital of Jilin University (No. 2019040806).

INFORMED CONSENT Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

RESEARCH REGISTRATION UNIQUE IDENTIFYING NUMBER (UIN) The unique identifying number is: researchregistry10330, and it is possible to find the registration here: https://www.researchregistry.com/browse-the-registry#home/.

GUARANTOR All authors have read and agreed to the published version of the manuscript.

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