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ORIGINAL RESEARCH

The Sex and Age-Associated Infiltration of B Cells May Result in the Dimorphic Behaviors Observed in Papillary Thyroid Carcinomas

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Background and Purpose: Sex and age show a dimorphism role in the pathogenesis, lymph node metastasis, and prognostic outcomes of papillary thyroid carcinoma. This investigation endeavors to elucidate the mechanisms underlying these disparities.

Methods: The clinicopathological characteristics and risk factors of lymph node metastasis were explored by analyzing the 2261 patients. The gene expression information of 497 samples from The Cancer Genome Atlas Thyroid Cancer database was used to explore the differentially expressed genes in different phenotypes. What's more, the single-cell RNA sequencing data obtained from the Gene Expression Omnibus database was used to explore the gene expression in specific cells.

Results: Multivariate logistic regression analysis showed that in male patients, a larger tumor size, extrathyroidal extension, younger age, and the presence of calcification emerged as significant predictors for lymph node metastasis (LNM)($p < 0.05$). Conversely, female patients exhibited a different profile, with larger tumor size, extrathyroidal extension, younger age, calcification, and bilateral tumors being identified as key risk factors ($p < 0.05$). Further stratification by age demonstrated distinct patterns: among the younger cohort, a larger tumor size, extrathyroidal extension, male gender, calcification, multifocality, and the presence of Hashimoto's thyroiditis held statistical significance ($p < 0.05$). In contrast, the older subgroup was characterized by a larger tumor size, extrathyroidal extension, male gender, calcification, bilateral tumors, and unclear margins as salient indicators of risk ($p < 0.05$). In the bulk gene analysis, there were two sex-age-related differentially expressed genes with a contrary trend in tissue sources and LNM status: TCL1A and CR2. The analysis of single-cell RNA sequencing showed that the infiltration of *TCL1A*- and *CR2*-related B cells varied in different clinical subtypes.

Conclusion: Lymph node metastasis of papillary thyroid carcinoma in different sexes and ages may have distinct patterns, and the ages-sex-related B cell infiltration might explain the dimorphism biological behavior.

Keywords: papillary thyroid carcinoma, lymph nodes metastasis, sex, age, immune microenvironment

Introduction

Papillary thyroid carcinoma (PTC), a prevalent form of malignant endocrine neoplasm, has seen a global upsurge in its incidence over recent years.[1,2] The thyroid gland is one of the largest endocrine glands in the human body, weighing 20–30 g in adults, and the thyroid lesions are often found, with a prevalence of 4%–7%, then most of them are asymptomatic, and with a normal hormone secretion function.[3] The prevalence of PTC among women is triple that of men, though older individuals continue to represent a demographic with a notably high incidence, there has been a discernible upswing in younger patients afflicted with PTC in recent years.[4,5] PTC exhibits a notably elevated incidence of lymph node metastasis (LNM), serving as both an aggressive biomarker and a potent predictor of tumor prognosis,[6,7] and some research has identified that a lower age and being male are significant risk factors for lymph node metastasis.[8,9] Furthermore, the male cohort exhibited a greater prevalence of aggressive clinical manifestations, including those

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characterized by vascular transgression, capsule invasion, and histologically invasive subtypes.[10,11] The younger and female patients tend to exhibit a more favorable prognosis. However, this advantage appears to diminish following the onset of menopause,[12,13] but younger patients were propensity to recurrence following surgical intervention.[12,13] What's more, a study examining the dynamic observation of papillary thyroid microcarcinomas has indicated that tumor progression appears to be more pronounced in younger individuals. Despite this, the intricate internal mechanisms responsible for this paradoxical and heterogeneous phenomenon remain shrouded in mystery.

Though the prognosis of PTC in an early stage is satisfactory,[14] part of the patients still die from metastatic advanced cancer. Troublesomely, some patients are resistant to the treatment of radioactive iodine (^{131}I), and only a few are sensitive to the targeted treatment.[15–17] Consequently, our quest for deeper understanding necessitates an expansion of our knowledge base to unravel the intricate biological behaviors of papillary thyroid carcinoma, thereby enabling us to make more judicious therapeutic decisions. The objective of this research is to delve into the fundamental mechanisms driving this clinical manifestation, with the ultimate goal of devising enhanced treatment strategies.

Materials and Methods

Clinical Data

This study presents a retrospective analysis encompassing a cohort of 2261 patients who, between 2014 and 2023, were diagnosed with PTC and subsequently underwent surgical intervention under the care of a single surgeon. Notably, at our institution, a standard protocol mandates the execution of routine prophylactic central compartment lymph node dissection for all confirmed PTC cases. Furthermore, the pathological evaluations were meticulously conducted by two seasoned pathologists, each independently assessing the specimens to ensure diagnostic accuracy. This study passed the ethical review based on the Declaration of Helsinki and was approved by the Ethics Committee of Tianjin Medical University General Hospital, and consent was obtained from each patient or subject after a full explanation of the purpose and nature of all procedures used. Inclusion criteria were: 1) initial PTC surgery; 2) completed clinical and pathological data; Exclusion criteria were: 1) recurrent PTC; 2) incidental PTC without central lymph node dissection (Figure 1). Then, 368 patients performed flow cytometry analysis to label lymphocyte subsets in peripheral blood.

The cut-off age was 45 years, which was the average age of all participants and lined with the age stratification of the 7th edition AJCC guidelines. Clinical features were defined as follows: sex (male or female), ultrasonogram features: aspect ratio (height divided by width, less than 1 or more than 1), margin (clear or unclear), and calcification (absent or present); preoperative serum assay: TSH (less than 2uIU/mL or more than 2uIU/mL) and serum thyroglobulin (less than 40 ng/mL or more than 40 ng/mL); postoperative pathology: tumor size (less than 1cm, 1cm-2cm, more than 2cm), multifocality (absent or present), bilateral tumor (absent or present), extrathyroidal extension (no capsule contacting, invading capsule and violating surrounding tissues), Hashimoto's thyroiditis (absent or present), nodular goiter (absent or present) and LNM (absent or present).

Public Data of Gene Expression

We downloaded the bulk gene expression data and clinical data of PTC from the TCGA database UCSC (https://xenabrowser.net/) and deleted the cases without LNM information. Further, 497 cases with completed age and sex information were selected, and the included genes were normally detected in at least 75% of participants. scRNA-seq data were extracted from the Gene Expression Omnibus (GEO) dataset (GSE184362),[18] and the quality control was performed according to the standard Seurat process.

Statistical Analysis and Immune Infiltration Evaluation

The SPSS 22.0 software was used for analyzing the statistical data. The continuous measurement data were expressed as mean average and standard deviation (± S) and univariate logistic regression analysis was performed for single-factor analysis. Multivariate analysis was performed by multivariate logistic regression analysis. A P value <0.05 indicated a statistically significant difference. The DEGs were screened from the TCGA database using the "DESeq2" package in R 4.2.2 software, which was defined as the average gene expression ratio and two times the standard deviation, with P <0.05.

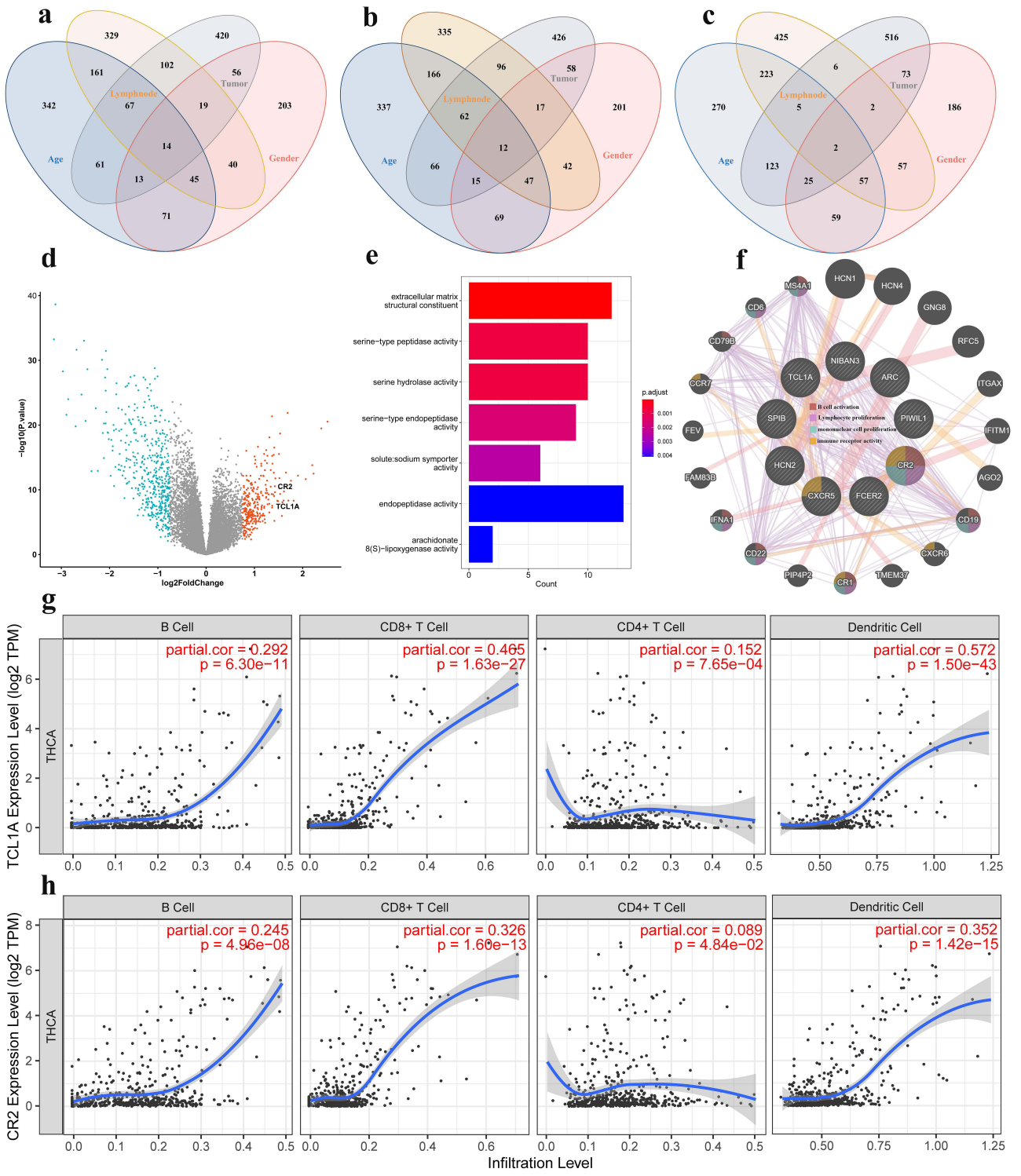

Figure 1 **(a)** Intersection of all DEGs in four contrast sequences; **(b)** Intersection of the sex-age related DEGs with the same regulated trend between lymph node metastasis status and tissue sources; **(c)** Intersection of sex-age related DEGs with the opposite trend between lymph node metastasis status and tissue sources; **(d)** Two sex-age related sharing DEGs with the opposite trend between lymph node metastasis status and tissue sources; **(e)** Go enrichment analysis of the same trend DEGs between lymph node metastasis status and tissue sources; **(f)** Protein-Protein Interaction Networks of the 9 sex-age related DEGs with the opposite trend between lymph node metastasis status and tissue sources in GENEMANIA database; **(g** and **h)** The correlation between the expression of *TCL1A* and *CR2* to immune cell infiltration evaluated in TIMER 2.0 Web Tools.

Results

Baseline Characteristics and Risk Factors of LNM in Overall Participants

Based on the clinical features, the 2261 subjects were divided as follows: 599 were males and 1662 were females, with a male-to-female ratio of 1:2.77; the average age of males was 44.2, and 45.2 for females; there were 1210 (53.5%) cases without LNM and 1051 (46.5%) cases with LNM. Univariate logistic regression analysis showed that in overall participants, sex (OR=1.879, 95% CI: 1.549–2.280, P<0.05), age (OR=0.520, 95% CI: 0.440–0.615, P<0.05), tumor size (P<0.05), multifocality (OR=1.460, 95% CI: 1.229–1.736, P<0.05), bilateral tumor (OR=1.825, 95% CI: 1.491–2.233, P<0.05), capsule invasion (P<0.05), margin on ultrasonogram (OR=1.264, 95% CI: 1.060–1.507, P<0.05), calcification (OR=1.993, 95% CI: 1.644–2.415, P<0.05), aspect ratio (OR=0.759, 95% CI: 0.642–0.898, P<0.05), nodular goiter (OR=0.801, 95% CI: 0.676–0.949, P<0.05), and serum thyroglobulin (OR=1.413, 95% CI: 1.151–1.734, P<0.05) had significant associations (P<0.05) with LNM (Table 1). Based on the above univariate analysis, the risk factors were enrolled in multivariate logistic regression. The Results showed the larger size (P<0.05), extra-thyroidal extension (P<0.05), male sex (OR=1.853, 95% CI: 1.509–2.276, P<0.05), younger age (OR=2.067, 95% CI: 1.731–2.469, P<0.05), calcification (OR=1.657, 95% CI: 1.351–2.033, P<0.05) and bilateral tumor (OR=2.158, 95% CI: 1.635–2.848, P<0.05) were independent risk factors for LNM (Table 2).

Table 1 Clinicopathological Characteristics and Univariate Analysis of the 2261 PTC Patients

Parameter	LNM(+) (1210)	LNM(-) (1051)	OR (95% CI)	P
Gender			1.879 (1.549–2.280)	0.000
Female	822	840		
Male	388	211		
Age (years)			0.520 (0.440–0.615)	0.000
<45	714	450		
≥45	496	601		
Tumor size (cm)				0.000
≤1	454	606		
1–2	516	337	2.044 (1.701–2.455)	0.000
>2	240	108	2.966 (2.293–3.838)	0.000
Aspect ratio			0.759 (0.642–0.898)	0.001
≤1	551	408		
>1	659	643		
Calcification			1.993 (1.644–2.415)	0.000
Absent	235	341		
Present	975	710		
Margin			1.264 (1.060–1.507)	0.009
Clear	367	373		
Unclear	843	678		
TSH			1.031 (0.874–1.217)	0.714
≤2uIU/mL	625	551		
>2uIU/mL	585	500		
Tg			1.413 (1.151–1.734)	0.001
≤40ng/mL	921	860		
>40ng/mL	289	191		
Multifocality			1.460 (1.229–1.736)	0.000
Absent	715	713		
Present	495	388		
Bilateral tumor			1.825 (1.491–2.233)	0.000
Absent	869	865		

(*Continued*)

Table 1 (Continued).

Parameter	LNM(+) (1210)	LNM(-) (1051)	OR (95% CI)	P
Present	341	186		
ETE				0.000
No capsule contacting	256	342		
Capsule invading	703	568	1.653 (1.359–2.012)	0.000
Violating surrounding tissues	251	141	2.378 (1.830–3.091)	0.000
Hashimoto's thyroiditis			0.851 (0.710–1.019)	0.080
Present	872	722		
Absent	338	329		
Nodular goiter			0.801 (0.676–0.949)	0.010
Present	436	434		
Absent	774	617		

Abbreviations: PTC, papillary thyroid carcinoma; ETE, extra thyroidal extension; LNM, lymph node metastasis; OR, Odds Ratio.

Table 2 Predictive Factors of LNM in PTC Patients in Multiple Logistic Regression Analysis

Parameter	p	Adjusted OR	95% CI for Adjusted OR	
			Lower	Upper
Sex	0.000	1.853	1.509	2.276
Age	0.000	2.067	1.731	2.469
Tumor size (cm)	0.000			
1–2	0.000	1.812	1.492	2.200
>2	0.000	2.574	1.962	3.376
ETE	0.000			
Capsule invading	0.000	1.652	1.343	2.033
Violating surrounding tissues	0.000	2.158	1.635	2.848
Calcification	0.000	1.657	1.351	2.033
Bilateral tumor	0.000	2.158	1.635	2.848
Constants	0.000	0.192	——	——

Abbreviations: LNM, lymph nodes metastasis; PTC, papillary thyroid carcinoma; ETE, extrathyroidal extension.

Characteristics and Risk Factors of LNM in Different Subgroups

In males, there were 121(25.3%) patients with a larger size tumor(>2cm) versus 227(20.9%) in females ($P < 0.05$). In addition, in males, there was a higher rate of nodular goiter background, a higher level of TSH, and a lower rate of Hashimoto's thyroiditis compared to females ($P < 0.05$). Moreover, the aspect ratio and calcification rate of the tumor are different in different sexes ($P < 0.05$). Comparing the differences in different age levels, we find that the younger were more likely to occur LNM and with a background of Hashimoto's thyroiditis and a lower level of serum thyroglobulin ($P < 0.05$). Moreover, the aspect ratio and the margin morphology of the tumor are different at different age levels ($P < 0.05$). The remaining features are shown in Table 3.

In males, 388 (64.8%) of all 599 patients had LNM, versus 822 (49.5%) of 1662 cases in females, indicating a statistically significant difference ($P < 0.05$). In males, age, tumor size, capsule invasion, bilateral tumor, calcification, and serum thyroglobulin had significant associations ($P<0.05$) with LNM. In females, age, tumor size, aspect ratio, multifocality, bilateral tumor, capsule invasion, Hashimoto's thyroiditis, calcification, and serum thyroglobulin had significant associations ($P<0.05$) with LNM (Table 4). For the younger subgroup, sex, aspect ratio of the tumor, tumor

Table 3 Clinicopathological Characteristics of PTC of the 2261 Patients in Different Subgroups

	Male (n=599)	Female (n=1662)		<45 years (n=1164)	≥45 years (n=1097)	
Parameter	**No.(Rate%)**	**No. (Rate%)**	**P**	**No. (Rate%)**	**No. (Rate%)**	**P**
Age (year)	(44.2±12.7)	(45.2±12.6)	0.076	——	——	——
<45	327(54.6)	837(50.4)		——	——	
≥45	272(45.4)	825(49.6)		——	——	
Aspect ratio			0.012			0.006
≤1	280(46.7)	679(40.9)		526(45.2)	433(39.5)	
>1	319(53.3)	983(59.1)		638(54.8)	664(58.7)	
Calcification			0.024			0.110
Absent	132(22.0)	444(26.7)		280(24.1)	296(27.0)	
Present	467(78.0)	1218(73.3)		884(75.9)	801(73.0)	
Margin			0.608			0.000
Clear	191(31.9)	549(33.0)		337(29.0)	403(36.7)	
Unclear	408(68.1)	1113(67.0)		827(71.0)	694(63.3)	
TSH	2.03±2.29	2.56±4.19	0.000	2.44±4.55	2.41±2.77	0.580
≤2 uIU/mL	372(62.1)	804(48.4)		612(52.6)	564(51.4)	
>2 uIU/mL	227(37.9)	858(51.6)		552(47.4)	533(48.6)	
Tg			0.801			0.004
≤40ng/mL	474(79.1)	1307(78.6)		945(81.2)	836(76.2)	
>40ng/mL	125(20.9)	355(21.4)		219(18.8)	261(23.8)	
Tumor size			0.001			0.826
≤1(cm)	266(44.4)	794(47.8)		547(47.0)	513(46.8)	
1–2(cm)	212(35.4)	641(38.6)	0.904	443(38.1)	410(37.4)	0.737
>2(cm)	121(20.2)	227(13.6)	0.000	174(14.9)	174(15.8)	0.548
Multifocality			0.339			0.024
Absent	388(64.8)	1040(62.6)		761(65.4)	667(60.8)	
Present	211(35.2)	622(37.4)		403(34.6)	430(39.2)	
Bilateral tumor			0.966			0.055
Absent	459(76.6)	1275(76.7)		912(78.3)	822(74.9)	
Present	140(23.4)	387(23.3)		252(21.7)	275(25.1)	
ETE			0.583			0.506
No capsule contacting	164(27.4)	434(26.1)		319(27.4)	279(25.4)	
Invading capsule	326(54.4)	945(56.9)	0.303	650(55.8)	621(56.6)	0.713
Violating surrounding tissues	109(18.2)	283(17.0)	0.517	195(16.8)	197(18.0)	0.449
Hashimoto's thyroiditis			0.000			0.001
Absent	528(88.1)	1066(64.1)		784(67.4)	810(73.8)	
Present	71(11.9)	596(35.8)		380(32.6)	287(26.2)	
Nodular goiter			0.000			0.001
Absent	150(25.0)	720(43.3)		487(41.8)	383(34.9)	
Present	449(75.0)	942(56.7)		677(58.2)	714(65.1)	
LNM			0.000			0.000
Absent	211(35.2)	840(50.5)		450(38.7)	601(54.8)	
Present	388(64.8)	822(49.5)		714(61.3)	496(45.2)	

Abbreviations: PTC, papillary thyroid carcinoma; ETE, extra thyroidal extension; LNM, lymph node metastasis; Tg, Thyroglobulin.

size, capsule invasion, multifocality, bilateral tumor, calcification, nodular goiter, and serum thyroglobulin had significant associations (P<0.05) with LNM. In the older subgroup, sex, tumor size, multifocality, bilateral tumor, capsule invasion, margin on ultrasonogram, and calcification had significant associations (P<0.05) with LNM (Table 5).

Table 4 Univariate Logistic Analysis of Risk Factors of LNM in Different Sex

	Male (n=599)			Female (n=1662)		
Parameter	**LNM(+) (n=388)**	**LNM(-) (n=211)**	**P**	**LNM(+) (n=822)**	**LNM(-) (n=840)**	**P**
Age (year)			0.000			0.000
<45	235	92		479	358	
≥45	153	119		343	482	
Aspect ratio			0.099			0.016
≤1	191	89		360	319	
>1	197	122		462	521	
Calcification			0.000			0.000
Absent	63	69		172	272	
Present	325	142		650	568	
Margin			0.294			0.019
Clear	118	73		249	300	
Unclear	270	138		573	540	
TSH			0.485			0.344
≤2uIU/mL	237	135		388	416	
>2uIU/mL	151	76		434	424	
Tg			0.012			0.015
≤40ng/mL	295	179		626	681	
>40ng/mL	93	32		196	159	
Tumor size(cm)			0.000			0.000
≤1	137	129		317	477	
1–2	154	58	0.000	362	279	0.000
>2	97	24	0.000	143	84	0.000
Multifocality			0.064			0.000
Absent	241	147		474	566	
Present	147	64		348	274	
Bilateral tumor			0.008			0.000
Absent	284	175		585	690	
Present	104	36		237	150	
ETE			0.000			0.000
No capsule contacting	86	78		170	264	
Capsule invading	219	107	0.002	484	461	0.000
Violating surrounding tissues	83	26	0.000	168	115	0.000
Hashimoto's thyroiditis			0.113			0.741
Present	348	180		524	542	
Absent	40	31		298	298	
Nodular goiter			0.224			0.272
Present	91	59		345	375	
Absent	297	152		477	465	

Abbreviations: LNM, lymph node metastasis; PTC, papillary thyroid carcinoma; Tg, Thyroglobulin; ETE, extra thyroidal extension.

Multi-Factor Analysis of LNM in Subgroups

Based on the above univariate analysis, in overall participants, risk factors that may be associated with LNM (P<0.05) including sex, age, tumor size, multifocality, bilateral tumor, capsule invasion, the margin on ultrasonogram, calcification, aspect ratio, nodular goiter, and serum thyroglobulin were enrolled in multivariate logistic regression. The results showed the larger size (P<0.05), extra-thyroidal extension (P<0.05), male sex (OR=1.853, 95% CI: 1.509–2.276, P<0.05), younger age (OR=2.067, 95% CI: 1.731–2.469, P<0.05), calcification (OR=1.657, 95% CI: 1.351–2.033, P<0.05) and bilateral tumor (OR=1.638, 95% CI: 1.321–2.030, P<0.05) were independent risk factors for LNM. In

Table 5 Univariate Logistic Analysis of Risk Factors of LNM in Different Age Levels

	<45 years (n=1164)			≥45 years (n=1097)		
Parameter	**LNM(+) (n=714)**	**LNM(-) (n=450)**	**P**	**LNM(+) (n=496)**	**LNM(-) (n=601)**	**P**
Sex			0.000			0.000
Male	235	92		153	119	
Female	479	358		343	482	
Aspect ratio			0.010			0.164
≤1	344	182		207	226	
>1	370	268		289	375	
Calcification			0.000			0.000
Absent	130	150		105	191	
Present	584	300		391	410	
Margin			0.762			0.002
Clear	209	128		158	245	
Unclear	505	322		338	356	
TSH			0.579			0.224
≤ 2 uIU/mL	380	232		245	319	
>2 uIU/mL	334	218		251	282	
Tg			0.000			0.319
≤40 ng/mL	550	395		371	465	
>40 ng/mL	164	55		125	136	
Tumor size (cm)			0.000			0.000
≤1	270	277		184	329	
1–2	305	138	0.000	211	199	0.000
>2	139	35	0.000	101	73	0.000
Multifocality			0.006			0.000
Absent	445	316		270	397	
Present	269	134		226	204	
Bilateral			0.000			0.000
Absent	534	378		335	487	
Present	180	72		161	114	
ETE			0.000			0.000
No capsule contacting	154	165		102	177	
Capsule invading	421	229	0.000	282	339	0.013
Violating surrounding tissues	139	56	0.000	112	85	0.000
Hashimoto's thyroiditis			0.007			0.603
Absent	502	282		370	440	
Present	212	168		126	161	
Nodular goiter			0.003			0.155
Absent	274	213		162	221	
Present	440	237		334	380	

Abbreviations: LNM, lymph nodes metastasis; PTC, papillary thyroid carcinoma; Tg, Thyroglobulin; ETE, extrathyroidal extension.

males, the results showed that the larger size (P<0.05), extra-thyroid extension (P<0.05), younger age (OR=1.885, 95% CI= (1.317–2.699);P< 0.05), and calcification (OR=2.097, 95% CI= (1.377–3.194) P<0.05) were independent risk factors. In the subgroup females, the larger size (P<0.05), extra-thyroid extension (P<0.05), younger age (OR=2.117, 95% CI: 1.725–2.600; P<0.05), calcification (OR=1.544, 95% CI= (1.223–1.950) P<0.05) and bilateral tumor (OR=1.713, 95% CI:1.342–2.186; P<0.05) were independent risk factors (Table 4). In the younger subgroup, the results showed that the larger size (P<0.05), extra-thyroid extension (P<0.05), male sex (OR=1.643, 95% CI= (1.215–2.222);P< 0.05), calcification (OR=1.943, 95% CI= (1.456–2.593) P<0.05), multifocality (OR=1.449, 95% CI= (1.106–1.897);P<

0.05) and Hashimoto's thyroiditis (OR=0.752, 95% CI= (0.572–0.990);P< 0.05) were independent risk factors. In older subgroup, the larger size (P<0.05), extra-thyroid extension (P<0.05), male sex (OR=1.919, 95% CI: 1.436–2.566; P<0.05), calcification (OR=1.377, 95% CI= (1.026–1.848) P<0.05), bilateral tumor (OR=1.736, 95% CI:1.298–2.323; P<0.05) and unclear margin (OR=1.341, 95% CI:1.027–1.750; P<0.05) were independent risk factors (Table 6).

TCGA Bulk Gene Expression and DEGs

Among the 497 samples, 444 were tumor tissues and 53 were para-tumor tissues. All participants were divided into different groups according to the different status of LNM, sex, and age, and the baseline was established according to the risk factors of LNM, showing: the female sex and the older age. The DEGs were defined as up-regulated, down-regulated, and unchanged, and Venn maps were constituted by intersecting DEGs in different contrast sequences. Among 202 sharing DEGs of tissue sources and LNM status, there were 100 sex-age related, and only 9 of them with a contrary regulated trend between tissue sources and LNM status (Figure 1a–c). In these contrary trend genes, only two were age and sex shared: *TCL1A* and *CR*, which was up-regulated in LNM cases (Figure 1d). Enrolling all same trend genes into Gene Ontology enrichment, the results showed a total of these 187 genes were mainly enriched in the structural constituent of extracellular matrix and serine-type peptidase activity (Figure 1f). The 9 contrary trend genes were analyzed by the GENEMANIA database to construct the protein-protein interaction network, then the results showed they functioned in immune-related pathways (Figure 1e).

Table 6 Predictive Factors of LNM in PTC Patients in Multiple Logistic Regression Analysis

	Male		Female	
Parameter	**p**	**Adjusted OR (95% CI)**	**p**	**Adjusted OR (95% CI)**
Sex	——	——	——	——
Age (year)	0.001	0.530(0.371–0.759)	0.000	0.472 (0.385–0.580)
Tumor size (cm)	0.000		0.000	
1–2	0.001	2.044 (1.365–3.062)	0.000	1.763 (1.413–2.200)
>2	0.000	3.070 (1.816–5.190)	0.000	2.456 (1.787–3.376)
ETE	0.002		0.000	
Invading capsule	0.006	1.770 (1.180–2.656)	0.000	1.629 (1.280–2.074)
Violating surrounding tissues	0.001	2.596 (1.479–4.556)	0.000	2.071 (1.503–2.852)
Calcification	0.001	2.097 (1.377–3.194)	0.000	1.544 (1.223–1.950)
Bilateral tumor	——	——	0.000	1.713 (1.342–2.186)
	<45 years		**≥45 years**	
Age	——	——	——	——
Gender	0.001	1.643 (1.215–2.222)	0.000	1.919 (1.436–2.566)
Tumor size (cm)	0.000		0.000	
1–2	0.000	1.981 (1.508–2.601)	0.000	1.719 (1.300–2.273)
>2	0.000	3.206 (2.103–4.887)	0.000	2.209(1.531–3.188)
ETE	0.000		0.003	
Invading capsule	0.000	1.875 (1.409–2.495)	0.014	1.460 (1.079–1.977)
Violating surrounding tissues	0.000	2.388 (1.603–3.588)	0.001	1.940 (1.313–2.866)
Multifocality	0.007	1.449 (1.106–1.897)	——	——
Hashimoto's thyroiditis	0.042	0.752 (0.572–0.990)	——	——
Margin	——	——	0.031	1.341 (1.027–1.750)
Calcification	0.000	1.943 (1.456–2.593)	0.033	1.377 (1.026–1.848)
Bilateral	——	——	0.000	1.736 (1.298–2.323)

Abbreviations: PTC, papillary thyroid carcinoma; LNM, lymph node metastasis; ETE, extra thyroidal extension.

TIMER Analysis of TCL1A and CR2

We explored two age-sex-related genes *TCL1A* and *CR2* by TIMER 2.0 Web Tools, which was used to evaluate the correlation between genes and immune cell infiltration. *TCL1A* had a moderate correlation with CD8+ (cor = 0.47, $p < 0.05$), and dendritic cells (cor = 0.57, $P < 0.05$). At the same time, *CR2* got a weaker correlation with CD8+ (cor = 0.33, $P < 0.05$), and dendritic cells (cor = 0.35, $P < 0.05$)(Figure 1g and h).

GEO Database of Single-Cell RNA Sequencing

We obtained the scRNA-seq data of 23 samples from the GSE184362, which were divided into four subgroups according to the status of LNM: tumor tissues with LNM, para-tumor tissues with LNM, tumor tissues without LNM and para-tumor tissues without LNM. Based on the expression of marker genes, all cells were assorted into the following types: thyroid cells (*TG, TPO*, and *TSHR*), T cells (*CD3D* and *CD3E*), B cells (*CD79A* and *CD79B*), and other undefined cells. Based on the marker genes, *TCL1A* and *CR2* were found mainly expressed in the B cell (*CD79A* and *CD79B*) (Figure 2a–d). We found that the expression of *TCL1A* and *CR2* were higher in para-tumor tissues and tissues with LNM than in tumor tissues without LNM (Figure 3a vs c and b vs d, Figure 3a vs b and c vs d).

We extracted the subsets of B cells by the marker genes *CD79A* and *CD79B* from a para-tumor tissue with LNM and divided it into *TCL1A*-Related cells and *TCL1A*-Non-Related cells. Based on the stage-specific genes of B-cell development, we constructed a pseudotime analysis map and a *TCL1A* gene expression map and listed the stage-specific genes (*CD74* and *MS4A1*) expression information, which showed that the expression of *TCL1A* correlated to the early stage B cells (Figure 4).

Peripheral Blood Lymphocyte Subpopulation

We sampled the peripheral blood of 368 patients with PTC for lymphocyte subsets classification. According to the complement on the surface of the lymphocyte, the cells were divided into *CD3+/CD4+* T lymphocytes, *CD3+/CD8+* T lymphocytes, and *CD19+* B lymphocytes. Our research found that the percent of *CD3+/CD8+*cells and the ratio of *CD3+/CD4+* to *CD3+/CD8+* in peripheral blood was higher in lymph node metastasis and the younger patients with a significantly different ($P<0.05$)(Figure 5).

Discussion

Our approach to thyroid cancer surgery is anchored in the Bethesda grading system derived from fine needle aspiration biopsies, offering a pivotal foundation for our clinical decisions regarding thyroid malignancies.[19,20] Previous studies have reported that age and sex performed a dimorphism role in the biological behavior of PTC.[21,22] Some studies speculated that the varied hormone levels and immune status in different sexes and ages should be responsible for these contradictory behaviors. In women, the levels of estrogen reached the peak in reproductive age and then decreased with the advent of menopause.[23–25] Moreover, estrogen and its receptors play an important role in autoimmune diseases including Hashimoto's thyroiditis, which frequently occurs in women especially in childbearing age.[26–28] Some studies found that Hashimoto's thyroiditis was a risk factor for PTC,[29,30] however, on the other hand, Hashimoto's thyroiditis was a protective factor for LNM,[31–33] and with Hashimoto's thyroiditis as a background patients got a better prognosis.[34] The changes in the immune microenvironment cause the pathogenesis of tumors, and the metastasis of lymph nodes to vary in different sexes and ages.

Our study found that females and younger were more likely to with a background of Hashimoto's thyroiditis, which seems to be a protective factor to LNM in younger ones. In addition, we found the expression of *TCL1A* and *CR2* were higher in females and the younger, which were up-regulated in tumor tissues and down-regulated in tumors with LNM. These contrary trends may explain the reason why the biological behavior of PTC was different in patients of different sexes and ages. The scRNA-seq analysis showed that *TCL1A* and *CR2* were mainly expressed in B lymphocytes, the infiltration of which was higher in tumor tissues with LNM, and all this evidence strengthened the influence of immunity on PTC. B cells play an important role in autoimmune diseases, not only by producing antibodies to participate in autoimmune reactions but also by secreting cytokines and chemokines to recruit T cells.[35]

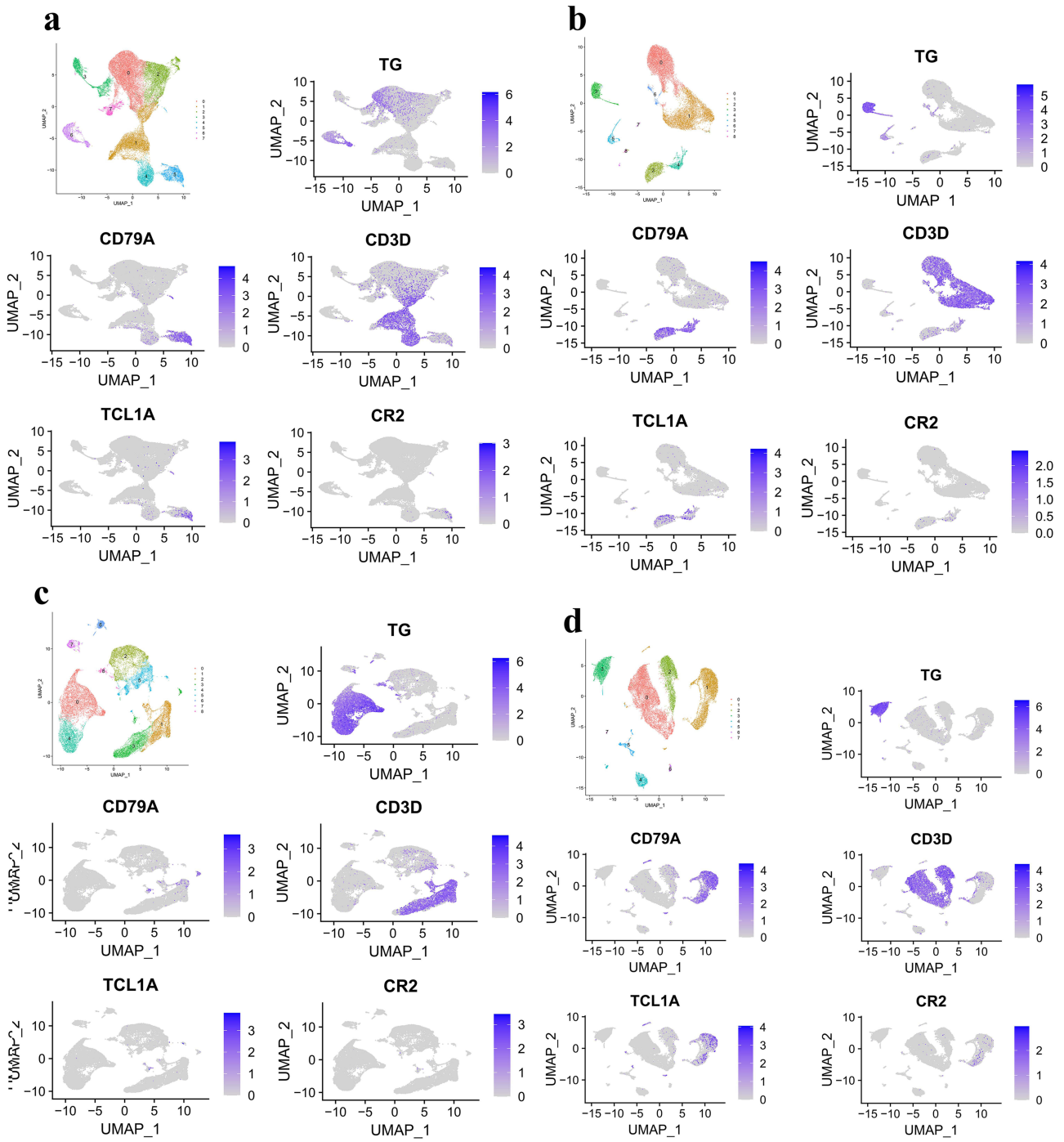

Figure 2 Clustering of cells and expression of marker genes. (**a**) Tumor tissue with lymph node metastasis; (**b**) Para-tumor tissue with lymph node metastasis; (**c**) Tumor tissue without lymph node metastasis; (**d**) Para-tumor tissue without lymph node metastasis.

TCL1A is a proto-oncogene, physiologically, which is only expressed in embryonic tissues and pre-mature B cells or early T cells[36] and is overexpressed in some T cell or B cell lymphomas and epithelial solid tumors.[37] The high expression of TCL1A was related to the LNM in breast cancer and the poorer prognosis in colon cancer.[38,39] TCL1A participated in the conversion of T and B lymphocytes by changing the expression of proinflammatory cytokines and chemokines, which promoted the development of autoimmune diseases.[40] Also, the expression of TCL1A in lymphocytes was induced by estrogen, which depended on single-nucleotide polymorphism.[41]

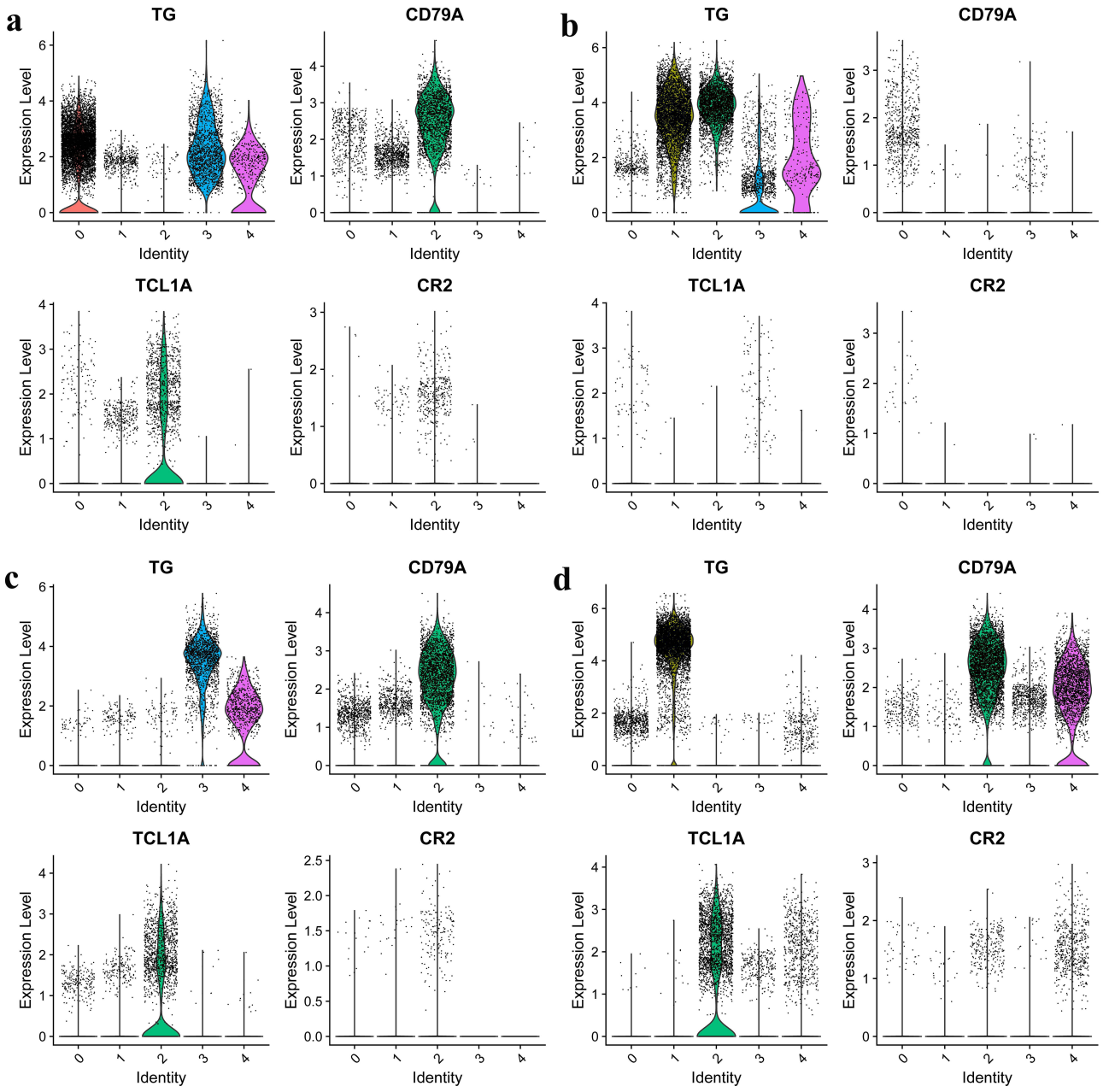

Figure 3 Expression of *TCL1A* and *CR2* in different tissues. (**a**) Tumor with lymph node metastasis; (**b**) Para-tumor tissue with lymph node metastasis; (**c**) Tumor without lymph node metastasis; (**d**) Para-tumor tissue without lymph node metastasis.

CR2 is a transmembrane glycoprotein expressed by mature B cells and dendritic follicular cells.[42] It acts as a complement receptor to bind C3d and cooperate with BCR and then participates in complement-induced immune response.[43] In human beings, the binding of BCR and CR2 is dose-dependent and inhibits the activation of B cells under a weak stimulation of BCR.[44] In addition, CR2 regulates the tolerance of B cells and participates in autoimmunity.[45–47] In tumor immunity, CR2 can combine with the Fc receptor to assist in receptor-mediated tumor killing.[48]

Our study found that the younger and the female patients had a higher infiltration of B cells in the tumor tissues with lymph node metastasis, but the tumor tissue got a lower infiltration than the para-tumor, the phenomenon of which represented whether LNM activates the tumor immune system by recruiting more lymphocytes. The influence of immune

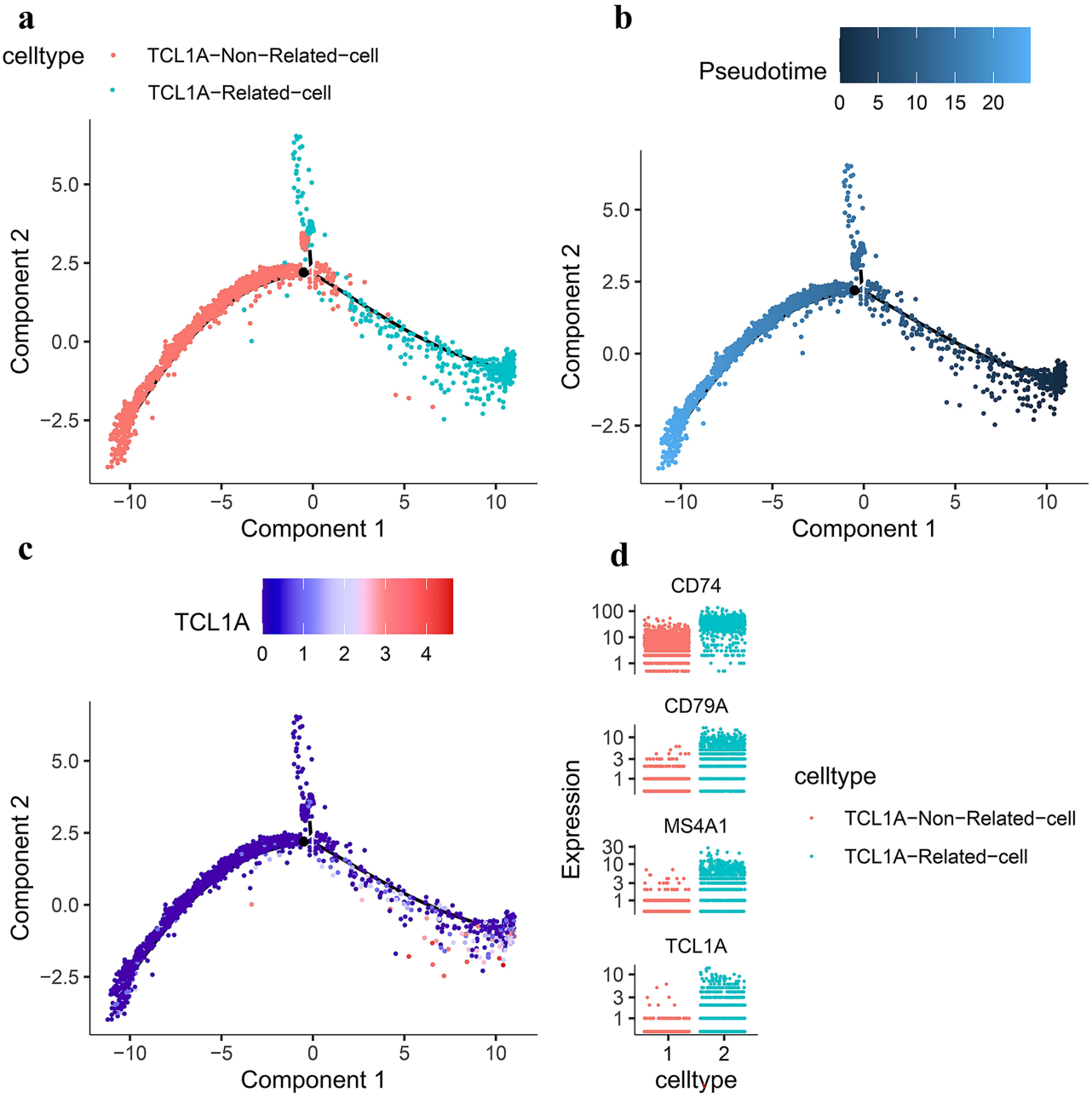

Figure 4 Clustering of B cells and pseudotime analysis of cells. (**a**) Two sub-clustering of B cells; (**b**) Pseudotime analysis of B cells; (**c**) The expression of TCL1A in B cells; (**d**) Expression of development-related genes in B-cell.

factors on the biological behavior of PTC has been confirmed, but the mechanism and pathway are complex and vague. Some studies on the single-cell RNA sequencing of PTC found different patterns of immune infiltration in PTC,[49] which affected the pathogenesis and metastasis of PTC. In addition, our study found a different proportion of CD3 + / CD8 + lymphocytes in peripheral blood with different lymph node states, which may help to evaluate the lymph node metastasis status before surgery.

However, our study was a single-center study, and hence the findings require further validation. Also, our study was conducted only on the transcription level. Hence, further proteomics research may help in obtaining more realistic results.

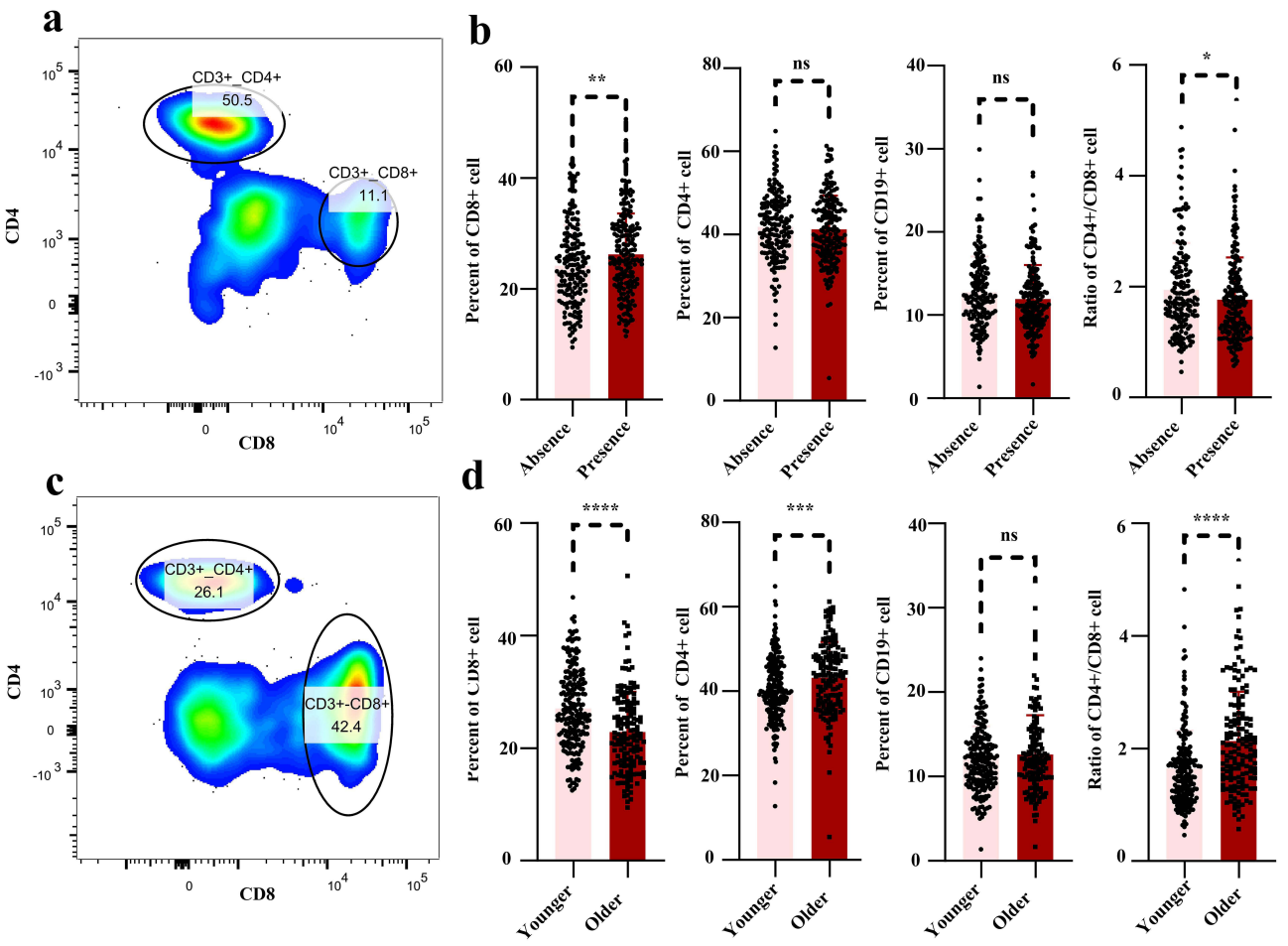

Figure 5 Peripheral blood lymphocyte subsets in patients with thyroid cancer. (**a**) Lymphocyte subsets in patients with lymph node metastasis; (**b**) Peripheral blood lymphocyte subsets with different lymph node metastatic status; (**c**) Peripheral blood lymphocyte subset in patients without lymph node metastasis; (**d**) Peripheral blood lymphocyte subsets with different age levels. **** P<0.0001, ***P<0.001,**P<0.01,*P<0.05, ns= not significant.

Conclusion

Our findings might help understand the role of sex and age in tumor pathogenesis and LNM of PTC, which is related to the expression of *TCL1A* and *CR2* in B cells. The results enriched our knowledge of immune factors in PTC. The infiltration of B cells for different ages and sexes might explain the contradictory biological behavior, including tumor pathogenesis, LNM, and prognosis of PTC.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflicts of interest in this work.

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