High Expression of Interleukin-12A and Its Association with the Clinicopathology and Prognosis of Differentiated Thyroid Cancer

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Keywords

Differentiated thyroid cancer · Interleukin-12A · Prognosis

Abstract

Background: The inflammatory microenvironment is closely related to the occurrence and development of cancer. Members of the interleukin-12 (IL-12) cytokine family play synergistic or antagonistic roles in the tumor microenvironment, in the form of classic heterodimers or newly discovered monomers or homodimers. **Objective:** The purpose of this study was to investigate the association between IL-12A and the clinicopathology and prognosis of differentiated thyroid cancer (DTC). **Methods:** A total of 101 pathologically confirmed DTC patients were included in this study. Immunohistochemistry was performed to assess IL-12A expression in DTC and corresponding paracancerous tissues. The associations of IL-12A with clinicopathology and prognosis were evaluated. **Results:** IL-12A was expressed in both normal thyroid tissues and DTC, but its expression level was significantly higher in DTC than in normal thyroid tissues ($p < 0.001$). IL-12A was positively correlated with tumor size ($p = 0.027$), risk stratification ($p = 0.020$), and TNM (Tumor-Node-Metastasis) stage ($p = 0.024$), but not with age, sex, patho-

logical type, multifocality, extrathyroid extension, lymph node metastasis, and distant metastasis (all $p > 0.05$). Lymphocytic thyroiditis was found in 26/101 patients (25.7%), which was negatively associated with IL-12A expression ($p = 0.018$). Multivariate logistic regression analysis showed that risk stratification was the significant independent predictor of IL-12A expression. The rate of disease persistence or recurrence (P&R) was 13/101 (12.9%), and a positive relationship was found between IL-12A expression and P&R ($p = 0.020$). Disease-free survival was affected by factors such as tumor size, extrathyroid extension, tumor stage (T stage), and IL-12A expression, with p values of 0.006, 0.048, 0.002, and 0.012, respectively. Multivariate Cox proportional-hazards analysis showed that tumor size ≥ 2 cm (hazard ratio [HR] = 4.041 [95% CI: 1.144–14.274], $p = 0.031$) and high IL-12A expression (HR = 4.027 [95% CI 1.014–15.994], $p = 0.049$) were independent predictors of prognosis of DTC patients. **Conclusions:** IL-12A is highly expressed in DTC and is associated with disease aggressiveness. In addition, IL-12A is an independent predictor of the outcome of DTC.

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Introduction

Thyroid cancer accounts for 1% of all human tumors and is the most common malignancy of the endocrine system [1]. In the past few decades, the incidence of this disease has sharply increased as a result of environmental factors and the rapid advances in diagnostic radiology tools [2]. Thyroid cancer is characterized by different clinicopathological properties, and differentiated thyroid cancer (DTC) accounts for more than 90% of thyroid cancer cases [3]. Although patients with DTC have a relatively good prognosis with standard treatments, 10–30% of patients suffer from persistence or recurrence (P&R), leading to high mortality rates [4].

A close relationship between inflammation and cancer was suggested by Virchow in 1863 [5]. Since then, the association between tumor formation and the inflammatory microenvironment has been repeatedly reported [6]. The tumor microenvironment of DTC is composed of a mixture of stromal cells, immune cells, and their secreted cytokines [7]. Furthermore, the components and structures of cytokines vary greatly, resulting in synergistic and antagonistic impacts of cytokines on the occurrence and development of cancer. For example, the proliferation and invasion of thyroid cancer are promoted by (C-X-C motif) ligand 10, but inhibited by (C-X-C motif) ligands 9 and 11 [8]. A large number of secreted proteins that bind to specific receptors and play a role in communication between leukocytes are referred to as interleukins (ILs) [9]. These cytokines are classified into different families according to sequence homology, receptor chain similarity, or functional characteristics [7]. The IL-12 family is a group of heterodimeric cytokines which consist of an α -chain (P19, P28, or P35) and a β -chain (P40 or Ebi3). The discovery of this cytokine family spans more than 50 years [10]. The IL-12 family includes IL-12, -23, -27, and -35, all of which are composed of a specific combination of an α -chain and a β -chain [11].

The roles of the IL-12 cytokine family are not fully understood. Researchers have focused on the antitumor effect of IL-12 or on the impacts of IL-12A (P35) and IL-12B (P40) gene polymorphisms on the progression of cancer [12–14]. The role of the IL-12 family on thyroid cancer was described in only one study, which reported a decrease in serum IL-12 expression in thyroid cancer compared with that in healthy controls; however, IL-12 expression was significantly higher in patients with active disease than in those who were disease free [15]. Few studies have reported an association between the IL-12 family and the prognosis of patients with cancer. There have been

reports demonstrating that the subunits of this cytokine family could exist and function as monomers and homodimers [16]. Therefore, the purpose of this study was to examine whether IL-12A is associated with clinicopathological features and whether this cytokine could act as a marker to predict the outcome of patients with DTC.

Subjects and Methods

Patients and Follow-Up

The present study was carried out at Shanghai Jiao Tong University Affiliated Sixth People's Hospital, a major ^{131}I treatment center in China. A total of 101 DTC patients were enrolled in the study according to the following inclusion criteria: (1) the patient had undergone total thyroidectomy, cervical lymph node dissection, and postoperative radioiodine treatment at our hospital between 2014 and 2015; (2) there was histopathological confirmation of papillary thyroid cancer or follicular thyroid cancer; (3) there was no history of other tumors and severe chronic diseases that affected survival, such as acquired immunodeficiency syndrome or kidney failure; and (4) the patient was followed up regularly. The standard follow-up protocol, which included free triiodothyronine, free thyroxine, thyroid-stimulating hormone, thyroglobulin (Tg), anti-Tg antibody, neck ultrasonography (US), chest computed tomography (CT) scans, and ^{131}I whole-body scans (^{131}I -WBS), was routinely performed.

Criteria for P&R of DTC

A patient was considered disease free if there was no imaging evidence by neck US and chest CT after surgery and by ^{131}I -WBS after radioiodine treatment, and if there was no serum evidence (Tg < 0.2 ng/mL) during the follow-up period after ^{131}I treatment. Active disease was defined as P&R of DTC in one of the following situations: (1) distant lesions were found and histopathologically confirmed as metastases of DTC during primary surgery; (2) distant lesions were detected by CT, were also positive in ^{131}I -WBS, and were accompanied by elevated serum stimulated Tg; (3) distant lesions were diagnosed by CT but were negative on ^{131}I -WBS, and were finally identified by fine needle aspiration biopsy or surgery; and (4) lesions were detected by US and accompanied by elevated serum Tg during follow-up, and were finally confirmed by fine needle aspiration biopsy or surgery. Disease-free survival (DFS) was defined as the time from the date of surgery to the diagnosis of distant metastasis or recurrence or to the date of the last follow-up. DFS was considered to be 0 when distant metastasis was present at the time of surgery [17].

Immunohistochemical Staining and Analysis

The most representative samples of 101 thyroid cancer and corresponding paracancerous tissues were selected to create a set of tissue microarrays with formalin-fixed, paraffin-embedded specimens, and the tissues at the same coordinate point of these microarrays in different slides were from the same patients. Immunohistochemical staining was performed on the tissue microarrays with a primary antibody against IL-12A (1:100 dilution, ab131039; Abcam) and was incubated overnight at 4 °C. The specimens were then incubated with horseradish peroxidase-conjugated goat anti-

rabbit secondary antibodies after having been rinsed with phosphate-buffered saline 3 times for 5 min each time. After 1-h incubation at room temperature, the slides were washed again with phosphate-buffered saline and were stained with diaminobenzidine; tap water was used to stop the staining process.

The dehydrated sections were restained with hematoxylin. Immunohistochemical staining was performed by two pathologists who were blinded to the patients' clinical data, each with a tissue microarray. The two stained slides were scanned with an automated digital slide scanner (Pannoramic MIDI 3DHISTECH) and were analyzed with the supporting analysis software QuantCenter. All the cells in the specimen were counted for positive rates, providing two positive rate reports for the specimens from the same patient. The final positive rate was the mean of the two. Low and high IL-12A expression was defined by the optimal positive rate cutoff value (70.7%). The threshold of 70.7% was defined by the X-tile software (version 3.6.1; Yale University, New Haven, CT, USA), which constructs a two-dimensional projection of each possible subgroup to show the robustness of the relationship between IL-12A and outcome, as previously described [18].

Statistical Analysis

Statistical analysis was performed using SPSS Statistics 21.0 (SPSS Inc., Chicago, IL, USA). A χ^2 test or Fisher's exact test was applied to evaluate the association between IL-12A expression and the clinicopathological characteristics and outcomes of DTC patients. The significant clinicopathological variables were then further analyzed with a multivariate logistic regression analysis model to define independent predictors of IL-12A expression. The DFS was compared using the Kaplan-Meier method, and the log-rank test was used to determine differences between two groups. Cox's proportional-hazards model was used to evaluate factors which affect DFS independently. The selection of prognostic factors, including in the analysis of the multivariate Cox regression model, was based on the clinical perspective, log-rank test results, and the reported factors potentially influencing survival. All tests were two-sided, and a p value <0.05 was considered statistically significant.

Results

IL-12A Overexpression in DTC and Correlation with Clinicopathological Characteristics

A total of 101 patients were retrospectively analyzed in the present study; the demographic and clinicopathological details on these patients are summarized in Table 1. An immunohistochemical assay was performed on the 101 cancer samples and the corresponding noncancerous tissues to evaluate the IL-12A expression levels in DTC. The mean rate of positivity for IL-12A was $49.0 \pm 24.6\%$ in the cancer group and $33.5 \pm 24.1\%$ in the normal group. A paired-samples t test showed a significant difference between the two groups ($p < 0.001$).

IL-12A was overexpressed in DTC compared with normal paracancerous tissues. The rate of positivity for IL-12A in DTC ranged from 0.29 to 91.86%, with a me-

Table 1. Demographics and clinicopathological features of the 101 enrolled patients with differentiated thyroid cancer

Age at diagnosis		
Mean $\pm$ SD (range), years	41.4 $\pm$ 14.1 (17–82)	
<55 years	77	76.2%
$\geq$ 55 years	24	23.8%
Sex		
Male	29	28.7%
Female	72	71.3%
Pathology of DTC		
FTC	14	13.9%
PTC	87	86.1%
T stage		
T1	55	54.5%
T2	12	11.9%
T3	16	15.8%
T4	18	17.8%
Lymph node metastasis		
No	25	24.8%
N1a	34	33.7%
N1b	42	41.6%
Distant metastasis		
No	93	92.1%
Yes	8	7.9%
Risk stratification		
Low risk	18	17.8%
Intermediate risk	77	76.2%
High risk	6	5.9%
TNM stage		
I	77	76.2%
II	20	19.8%
III	1	1.0%
IV	3	3.0%
Lymphocytic thyroiditis		
No	75	74.3%
Yes	26	25.7%

SD, standard deviation; DTC, differentiated thyroid cancer; FTC, follicular thyroid cancer; PTC, papillary thyroid cancer; T stage, tumor stage; TNM stage, Tumor-Node-Metastasis stage.

dian of 53.2%. The cutoff value for IL-12A positivity was defined as 70.7% using the X-tile software, based on the association of IL-12A with DFS in DTC patients, as shown in Figure 1a. Accordingly, the 101 patients were divided into two groups: a high IL-12A expression group, with a positivity rate $>70.7\%$, and a low IL-12A expression group, with a positivity rate $<70.7\%$. The expression levels of IL-12A in normal thyroid tissues, as well as in the low expression group and the high expression group, are shown in Figure 2.

The high IL-12A expression group consisted of 20 patients, including 7 male and 13 female patients, with a

Fig. 1. X-tile analysis of survival data for differentiated thyroid cancer patients reveals a continuous distribution based on the IL-12A positivity rate. **a** The plot shows that a cutoff value of 70.7% was defined by the X-tile data, which is represented in a rectangular-triangular grid. The vertical axis represents all possible “high IL-12A expression” populations, with the size of the high expression population increasing from top to bottom. Similarly, the horizontal axis represents all possible “low IL-12A expression” populations, with the size of the low expression population increasing from left to right. Data along the hypotenuse represent results from a single cut-point that divides the IL-12A expression rate into low

or high subsets. The strength of the inverse association between every cut-point of the IL-12A positivity rate and disease-free survival is assessed by a χ^2 value, shown by a color code in the grid, ranging from “low” (dark, black) to “high” (bright, red). The program can select the optimal cutoff value by selecting the highest χ^2 value, corresponding to the brightest region, marked by arrows. **b** The cut-point displayed on a histogram of the entire cohort. **c** Kaplan-Meier curve showing disease-free survival based on the IL-12A expression level (low expression subset: green; high expression subset: gray).

Fig. 2. Expression of IL-12A in normal thyroid tissues and thyroid cancer tissues detected by immunohistochemistry. **a** Expression of IL-12A in paracancerous tissues. **b** Low expression of IL-12A in thyroid cancer. **c** High expression of IL-12A in thyroid cancer.

Table 2. Correlations of IL-12A expression with clinicopathological characteristics and outcomes of patients with differentiated thyroid cancer

Variables	Total, <i>n</i>	IL-12A expression, <i>n</i>		χ^2	<i>p</i> value
		low	high		
Age					
<55 years	77	60	17	1.057	0.389
≥55 years	24	21	3		
Sex					
Male	29	22	7	0.482	0.488
Female	72	59	13		
DTC					
PTC	87	72	15	2.591	0.145
FTC	14	9	5		
Multifocality					
No	70	56	14	0.006	0.940
Yes	31	25	6		
Tumor size					
<2 cm	71	61	10	4.920	0.027
≥2 cm	30	20	10		
Extrathyroid extension					
No	76	63	13	1.406	0.256
Yes	25	18	7		
T stage					
T1–T2	67	56	11	1.435	0.231
T3–T4	34	25	9		
Lymph node metastasis					
No	25	21	4	0.302	0.774
Yes	76	60	16		
Distant metastasis					
No	93	76	17	1.714	0.192
Yes	8	5	3		
Risk stratification					
Low	18	18	0	5.408	0.020
Intermediate to high	83	63	20		
TNM stage					
I–II	97	80	17	7.991	0.024
III–IV	4	1	3		
Lymphocytic thyroiditis					
No	75	56	19	5.613	0.018
Yes	26	25	1		
Persistence or recurrence					
No	88	74	14	6.524	0.020
Yes	13	7	6		

Bold typeface denotes significance. IL-12A, interleukin-12A; DTC, differentiated thyroid cancer; FTC, follicular thyroid cancer; PTC, papillary thyroid cancer; T stage, tumor stage; TNM stage, Tumor-Node-Metastasis stage.

mean age of 36.2 ± 14.6 years (range 19–65). A total of 81 patients were in the low IL-12A expression group, including 22 males and 59 females, with a mean age of $42.7 \pm$

Table 3. Multivariate logistic analysis of clinicopathological characteristics influencing IL-12A expression

Variables	OR	95% CI	<i>p</i> value
Tumor size			
<2 cm	1		
≥2 cm	2.315	0.760–7.054	0.140
Risk stratification			
Low	1		
Intermediate to high	5.996	1.191–30.198	0.030
TNM stage			
I–II	1		
III–IV	0.886	0.378–2.075	0.780
Lymphocytic thyroiditis			
No	1		
Yes	0.132	0.016–1.095	0.061

Bold typeface denotes significance. IL-12A, interleukin-12A; TNM stage, Tumor-Node-Metastasis stage; OR, odds ratio; CI, confidence interval.

13.7 years (range 17–82). There was no significant difference in mean age between the two groups ($p = 0.063$). According to the American Joint Committee on Cancer TNM classification system (8th edition), the enrolled patients were divided into a young group (<55 years) and an old group (≥55 years). The association of the IL-12A expression level with clinicopathological features was first evaluated with a χ^2 test (Table 2). No significant associations were found between IL-12A expression and age, sex, pathological type, and multifocality (all $p > 0.05$; Table 2). IL-12A expression was negatively correlated with lymph node metastasis (lymphocytic thyroiditis [LT]; $p = 0.018$); however, multivariate logistic regression analysis showed that LT was no independent predictor of IL-12A expression ($p = 0.061$; Table 3).

Positive Association of IL-12A with Aggressiveness of DTC

The relationship between clinicopathological signs of aggressiveness and IL-12A expression was analyzed, as shown in Table 2. Although IL-12A was not associated with extrathyroid extension, cervical lymph node metastasis, and distant metastasis, it was positively associated with tumor size ($p = 0.027$), risk stratification level ($p = 0.020$), and TNM stage ($p = 0.024$). Multivariate logistic regression analysis defined risk stratification as the independent factor to predict the expression of IL-12A ($p = 0.030$, OR = 5.996 [95% CI: 1.191–30.198]; Table 3). Of the 101 patients, there were 13 (12.9%) suffering from P&R, of whom 2 patients were diagnosed with

Table 4. Univariate analysis of significant prognostic factors influencing disease-free survival of 101 patients with differentiated thyroid cancer

Variables	Patients, <i>n</i>	P&R, <i>n</i>	log-rank value	<i>p</i> value
Age				
<55 years	77	9	0.458	0.499
≥55 years	24	4		
Sex				
Male	29	4	0.055	0.815
Female	72	9		
Pathology of DTC				
FTC	14	3	1.160	0.282
PTC	87	10		
Multifocality				
No	70	7	1.605	0.205
Yes	31	6		
Tumor size				
<2 cm	71	5	7.511	0.006
≥2 cm	30	8		
Extrathyroid extension				
Yes	76	7	3.904	0.048
No	25	6		
T stage				
T1–T2	67	4	9.177	0.002
T3–T4	34	9		
Lymph node metastasis				
No	25	2	0.695	0.404
Yes	76	11		
TNM stage				
I–II	97	11	7.984	0.005
III–IV	4	2		
Lymphocytic thyroiditis				
No	75	9	0.184	0.668
Yes	26	4		
IL-12A expression				
Low	81	7	6.323	0.012
High	20	6		

Bold typeface denotes significance. IL-12A, interleukin-12A; DTC, differentiated thyroid cancer; FTC, follicular thyroid cancer; PTC, papillary thyroid cancer; background disease, Hashimoto thyroiditis or lymphocytic thyroiditis; T stage, tumor stage; TNM stage, Tumor-Node-Metastasis stage; P&R, persistence or recurrence.

criterion 1 mentioned above (in subsection Criteria for P&R of DTC), 3 patients with criterion 2, 3 patients with criterion 3, and 5 patients with criterion 4. The P&R group consisted of 4 males and 9 females, with a mean age of 42.8 ± 18.9 years (range 24–82). A statistical test found that IL-12A expression was significantly higher in the P&R group than in the non-P&R group ($p = 0.020$; Table 2).

High Expression of IL-12A Is an Independent Predictor of Poor DFS of Patients with DTC

DFS was defined as the time from the date of surgery to the diagnosis of distant metastasis or recurrence or the date of the last follow-up. The mean follow-up time was 44.8 ± 13.9 months (range 0–60). Three patients died during follow-up; 1 patient died of thyroid cancer and 2 died of tumors in other organs. DFS was significantly different between the two groups (13.6 ± 12.8 vs. 49.4 ± 5.7 months, $p < 0.001$), with shorter DFS in the P&R group than in the non-P&R group.

The Kaplan-Meier method and log-rank test were applied to assess the impact of the clinicopathological factors and the rate of IL-12A positivity on P&R of thyroid cancer. The results revealed that the rate of IL-12A positivity was associated with the DFS of DTC patients ($p = 0.012$; Fig. 1c; Table 4). Moreover, factors such as tumor size, extrathyroid extension, T stage, and TNM stage also affected DFS, with p values of 0.006, 0.048, 0.002, and 0.005, respectively. The details of the log-rank test and Kaplan-Meier curves are shown in Table 4 and Figure 3.

Multivariate Cox proportional-hazards analysis was performed to determine how strong the relationship was between IL-12A expression and the DFS of DTC patients. The results showed that there was still a strong association between tumor size, IL-12A expression, and DFS (all $p < 0.05$), after excluding the effect of other factors (Table 5). The risk of P&R was more than 4 times higher among patients with tumor sizes ≥ 2 cm than among those with tumor sizes < 2 cm ($p = 0.031$, HR = 4.041 [95% CI: 1.144–14.274]), and it was more than 4 times higher among patients with high IL-12A expression than among those with low IL-12A expression ($p = 0.049$, HR = 4.027 [95% CI: 1.014–15.994]). Therefore, a tumor size ≥ 2 cm and high IL-12A expression are considered independent predictors of worse outcome in DTC patients.

Discussion

The data on 101 patients were retrospectively analyzed. We found that IL-12A was expressed in both normal thyroid tissues and DTC tissues, while the expression of IL-12A was significantly enhanced in DTC specimens. IL-12A expression in thyroid cancer was not associated with clinicopathological features such as age, gender, pathological type, multifocality, extrathyroid extension, lymph node metastasis, and distant metastasis. High IL-12A expression was associated with aggressiveness of the DTC, as shown by the positive relationship of the IL-12A

Fig. 3. Kaplan-Meier curves of disease-free survival according to tumor size (a), extrathyroid extension (b), T stage (c), and pTNM stage (d).

positivity rate to malignant indicators such as tumor size, risk stratification, and TNM stage. LT was found in 25.7% of the DTC patients in the present study, which was lower than the rates in previous reports [19, 20]. This difference may be due to the small sample size, selection, and the regional bias of our study. Multivariate logistic analy-

sis defined risk stratification as an independent predictor of IL-12A expression. The frequency of P&R of DTC was 12.9%, which was similar to the rate of 11.4% that was reported by Trimboli et al. [21]. We showed that IL-12A was related to P&R of thyroid cancer. Furthermore, we demonstrated that the risk of P&R significantly increased

Table 5. Multivariate analysis of prognostic factors for disease-free-survival of 101 differentiated thyroid cancer patients using Cox's proportional-hazards model

Variables	HR	95% CI	<i>p</i> value
Age			
<55 years	1		
≥55 years	3.335	0.811–13.720	0.097
Sex			
Male	1		
Female	1.099	0.328–3.682	0.879
Pathology of DTC			
FTC	1		
PTC	0.215	0.030–1.545	0.129
Tumor size			
<2 cm	1		
≥2 cm	4.041	1.144–14.274	0.031
Multifocality			
No	1		
Yes	2.450	0.688–8.725	0.169
Extrathyroid extension			
No	1		
Yes	1.634	0.424–6.298	0.478
Lymph node metastasis			
No	1		
Yes	5.858	0.820–41.834	0.080
Lymphocytic thyroiditis			
No	1		
Yes	4.007	0.842–19.058	0.083
IL-12A expression			
Low	1		
High	4.027	1.014–15.994	0.049

Bold typeface denotes significance. IL-12A, interleukin-12A; DTC, differentiated thyroid cancer; FTC, follicular thyroid cancer; PTC, papillary thyroid cancer; HR, hazard ratio; CI, confidence interval.

in patients with high IL-12A expression, and IL-12A was an independent factor for predicting poor prognosis in patients with DTC.

The IL-12 family consists of a group of cytokines that have different impacts on the outcome of cancer, infection, and inflammatory diseases [10]. Chain sharing occurs extensively in this heterodimeric cytokine family and its receptors. All cytokines of the IL-12 family are composed of an α -chain and a β -chain, which are connected by a disulfide bond. The α -chain includes three components (P35, P19, and P28) and the β -chain includes two components (P40 and Ebi3). The P40 chain pairs with P35 or P19 to form IL-12 or IL-23, while Ebi3 pairs with P28 or P35 to form IL-27 or IL-35, respectively. The α -chain P35 (IL-12A) is a constituent of both IL-12 and

IL-35, which are two cytokines that have antagonistic effects on the tumor [16].

IL-12 was reported to be more highly expressed in tumor specimens than in the normal tissues of corresponding organs. In addition, it was well recognized as a cytokine that acts against tumors, with high expression predicting a superior outcome in cancer patients [22, 23]. However, the present study found that IL-12A was highly expressed in DTC and was associated with poor outcome. Part of the reason for the contradictory results is that IL-12A expression in this study simultaneously reflected the total expression of both IL-12 and IL-35, because IL-12A is a component of both of these cytokines. Whereas the expression of IL-12 was decreased in tumor tissues, the expression of IL-35 was significantly upregulated. Therefore, the high expression of IL-12A was mainly the result of IL-35 expression. Although the heterodimeric structure is a key feature of IL-12 family members, studies have reported that the subunits of this family can exist and function as monomers and homodimers. The results of this study suggest that IL-12A may exist in the tumor microenvironment as a monomer and as a homodimer, which competitively inhibit the effect of IL-12 or function as negative regulatory cytokines of immune responses against tumors. The exact structural forms, functions, and mechanisms of IL-12A in the DTC microenvironment need to be further explored in future studies.

In conclusion, the present study demonstrates that IL-12A is highly expressed in DTC. In addition, the IL-12A expression level is associated with the aggressiveness of the disease. Moreover, IL-12A could act as an independent predictive factor for the outcome of DTC.

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Statement of Ethics

The study protocol was approved by the Ethics Committee of Shanghai Jiao Tong University Affiliated Sixth People's Hospital. Written informed consent was obtained from the 101 enrolled patients.

Disclosure Statement

There is no conflict of interest to declare.

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Author Contributions

The study was designed by Q.-Y. Luo and Z.-L. Qiu; H.-J. Song collected the pathological tissue samples; G.-Q. Zhang and C.-T. Shen conducted the statistical analysis and wrote the paper.

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