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Original Article

Effects of ^{131}I and TSH suppression therapy on METTL3, METTL14 levels and recurrence in thyroid cancer

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Abstract: Objective: This study aims to evaluate the changes in the expression levels of METTL3 and METTL14 in patients with differentiated thyroid cancer (DTC) and their association with thyroid function indicators, as well as to explore the potential value of these genes in predicting the risk of DTC recurrence. Methods: This cohort study included 189 DTC patients treated at Shidong Hospital between April 2016 and February 2021. Patients were divided into an experimental group, which received combined radioactive iodine (^{131}I) therapy and thyroid-stimulating hormone (TSH) suppression therapy (n = 119), and a control group, which received only TSH suppression therapy (n = 70). Messenger RNA (mRNA) expression levels of METTL3 and METTL14 in patients' serum were measured before and six months after treatment using quantitative real-time polymerase chain reaction (qRT-PCR). Thyroid function indicators, including free triiodothyronine (FT3), free thyroxine (FT4), TSH, and thyroglobulin (Tg), were assessed using electrochemiluminescence immunoassay. Disease-free survival (DFS) was analyzed using Cox regression analysis, and data visualization was performed with the ggplot2 package in R. Results: Both METTL3 and METTL14 expression levels significantly decreased after treatment in both the experimental and control groups ($P < 0.001$). Regarding thyroid function indicators, FT3 and FT4 levels significantly increased, while TSH and Tg levels significantly decreased post-treatment ($P < 0.001$). Lower post-treatment expression levels of METTL3 and METTL14 were significantly associated with a higher risk of recurrence. Cox regression analysis further indicated that post-treatment METTL3, METTL14, TSH, and Tg levels were independent predictors of DFS ($P < 0.05$). Conclusion: Low expression levels of METTL3 and METTL14 are closely associated with malignant progression and an increased risk of recurrence in DTC. Patients receiving combined ^{131}I and TSH suppression therapy demonstrated longer DFS. These findings suggest that METTL3 and METTL14 could serve as potential biomarkers for prognosis evaluation in DTC patients.

Keywords: Thyroid cancer, ^{131}I therapy, TSH suppression therapy, METTL3, METTL14, postoperative recurrence

Introduction

In recent years, the incidence of thyroid cancer (TC) has been steadily increasing worldwide, particularly in countries such as the United States and China, raising widespread concern [1]. According to data from the National Cancer Institute (2023), the incidence of TC continues to rise globally, and by 2030, TC is expected to become the second most common cancer among women in the United States [2]. In China, the detection rate of TC has also significantly increased due to the widespread use of advanced screening techniques [3]. Although TC is generally considered an endocrine malignancy with a favorable prognosis, a considerable proportion of patients still face the risk of recurrence and distant metastasis after initial surgical treatment, which threatens their long-term survival and quality of life [4]. Consequently, the treatment of TC has become increasingly standardized with medical advancements.

Differentiated thyroid cancer (DTC), which includes papillary and follicular TCs, represents the most common and well-differentiated forms of TC [5]. These cancers are typically associated with a better prognosis due to their higher degree of cell differentiation. In contrast, other subtypes of TC include anaplastic and medul-

lary TCs, which are more aggressive and have worse clinical outcomes [5]. The standard treatment for DTC includes total or near-total thyroidectomy, followed by radioactive iodine (^{131}I) therapy and thyroid-stimulating hormone (TSH) suppression therapy [6]. The goal of ^{131}I therapy is to eliminate residual thyroid tissue and occult lesions, thereby reducing the risk of recurrence, particularly in early postoperative disease management [7]. TSH suppression therapy aims to reduce serum TSH levels, thereby decreasing the growth stimulus to thyroid cells and further inhibiting the growth of potential residual or recurrent tumors [8]. Although this combined treatment strategy is widely used in clinical practice, its specific effects and long-term impacts require further research and evaluation, especially in patients with varying risk levels, where treatment efficacy may differ [9]. Therefore, an in-depth exploration of factors influencing postoperative therapeutic responses in DTC patients is crucial for optimizing treatment protocols and improving patient prognosis.

METTL3 and METTL14 are key components of the N6-methyladenosine (m6A) methyltransferase complex, playing important biological roles in DTC [10]. METTL3, the main catalytic subunit of this complex, regulates post-transcriptional gene expression through m6A modification [11]. In DTC, low expression of METTL3 is closely associated with tumor cell dedifferentiation, increased malignancy, and accelerated tumor progression [12]. Additionally, METTL14, although lacking independent catalytic activity, acts as a cofactor of METTL3, enhancing the methyltransferase activity to stabilize RNA structure, which is crucial for maintaining the differentiation state of thyroid cells [13]. Studies have shown [14, 15] that dysfunction of METTL3 and METTL14 may lead to dedifferentiation and malignant progression of DTC cells, making these molecules significant in the pathogenesis of DTC and the development of new therapeutic strategies.

The novelty of this study lies in the first systematic evaluation of changes in the expression levels of METTL3 and METTL14 in patients with DTC and their relationship with thyroid function indicators. By exploring the roles of these two m6A-modifying enzymes in DTC cell differentiation and malignant progression, this study not only reveals their potential involvement in the pathogenesis of DTC but also provides new insights for personalized treatment of DTC.

Materials and methods

Sample size calculation

The sample size for this study was calculated based on recurrence rates reported in previous studies. Ye et al. [16] reported a recurrence rate of 10.5%, while Crepeau et al. [17] reported a rate of approximately 17%. Using the average recurrence rate from these two studies, we applied the following formula to determine the required sample size: $N = Z^2 \times [P \times (1\text{-}P)]/E^2$. With a confidence level of 95% ($Z = 1.96$), a margin of error (E) set at 5%, and an average recurrence rate (P) of 13.75%, the required sample size was calculated to be approximately 183 patients. Considering the practicalities of clinical sample collection, a total of 189 patients were included in the study, which provided sufficient power to detect significant differences in recurrence outcomes.

General information

This cohort study included 189 patients with DTC who were treated at Shidong Hospital between April 2016 and February 2021. All patients underwent total or subtotal thyroidectomy, followed by either radioactive iodine (^{131}I) therapy, TSH suppression therapy, or a combination of both. Specifically, 119 patients received combined ^{131}I and TSH suppression therapy and were designated as the experimental group, while 70 patients received only TSH suppression therapy and were designated as the control group. Additionally, a normal group comprising 25 healthy individuals who underwent routine check-ups at our hospital during the same period was included for baseline comparisons of METTL3 and METTL14 levels. This study has been approved by the ShidongHospital Ethics Committee.

Inclusion and exclusion criteria

Inclusion criteria for the study were: age over 18 years; undergoing total or subtotal thyroidectomy in accordance with the “TC Diagnosis and Treatment Guidelines” [18]; postoperative pathology confirming DTC; receipt of postoperative ^{131}I therapy and/or TSH suppression thera-

py; availability of METTL3 and METTL14 data before and after treatment; and complete clinical data. Exclusion criteria included: significant organ dysfunction, other malignancies, infectious diseases, hematologic diseases, or autoimmune diseases; history of using immunosuppressants, glucocorticoids, estrogens, or other relevant drugs within the past six months; pregnancy or lactation; history of neck surgery, radiotherapy, or iodine therapy; parathyroid injury during surgery; and rehospitalization due to recurrence.

Treatment protocol

All enrolled patients underwent total or subtotal thyroidectomy. Following surgery, patients were allocated to either an experimental or a control group. Patients in the control group received only TSH suppression therapy with levothyroxine sodium tablets (daily dose of 75-150 μg, taken on an empty stomach), maintaining serum TSH levels within the standard range (≤ 0.10 - < 0.05 mU/L, depending on staging). Serum TSH levels were rechecked every 4-6 weeks, and thyroid function was reassessed every six months, with medication doses adjusted as necessary. Patients in the experimental group additionally received an initial dose of 70-100 mCi of ^{131}I one month after surgery. After imaging confirmed no residual thyroid tissue, thyroid ablation was performed, and further ^{131}I therapy was administered based on treatment effectiveness. Both groups followed the same TSH suppression therapy protocol. Prior to ^{131}I therapy, patients were instructed to discontinue parathyroid hormone supplements and avoid iodine-containing drugs/foods and iodine contrast agents for 2-4 weeks.

Data collection

Clinical data of the 189 DTC patients were collected from the hospital's laboratory information system and electronic medical record system. Variables included age (years), gender, body mass index (BMI; kg/m^2), clinical stage, T stage, N stage, M stage, tumor diameter (cm), disease type (papillary vs. follicular), surgical method (total resection vs. subtotal resection), number of lesions, as well as the expression levels of METTL3 and METTL14 before and after treatment, and thyroid function indicators including free triiodothyronine (FT3; pg/mL), free thyroxine (FT4; ng/dL), TSH (μIU/mL), and thyroglobulin (Tg; ng/mL). "Before treatment" refers to measurements taken prior to surgical treatment, and "after treatment" refers to measurements taken six months post-treatment.

qRT-PCR detection

We extracted total RNA from patients' serum samples using Trizol reagent and reverse transcribed it into cDNA for qRT-PCR amplification. The reaction system had a total volume of 20 μL, which included cDNA, specific primers, and SYBR® Premix Ex Taq™ II. The PCR amplification process involved 40 cycles with a denaturation step at 95°C and annealing at 60°C. GAPDH was used as the internal reference gene, and the relative expression levels of METTL3 and METTL14 were calculated using the 2-ΔΔCt method. The primer sequences were as follows: METTL3 (forward: 5'-AAGCTGCACTTCAGACGAAT-3', reverse: 5'-GGAATCACCTCCGACACTC-3'), METTL14 (forward: 5'-AGTGCCGACAGCATTGGTG-3', reverse: 5'-GGAGCAGAGGTATCATAGGAAGC-3'), and GAPDH (forward: 5'-GCTGTAGCCAAATCGTTGT-3', reverse: 5'-CCAGGTGGTCTCCTCTGA-3').

Thyroid function testing

Thyroid function was evaluated using electrochemiluminescence immunoassay to measure serum levels of FT3, FT4, TSH, and Tg. The Roche Diagnostics Cobas e411 automatic immunoassay analyzer was employed, ensuring high sensitivity and specificity in measuring the thyroid function indicators. All samples were tested according to the manufacturer's instructions to ensure accuracy and consistency of the results.

BEST platform analysis

The Bioinformatics & Evolutionary Studies (BEST) platform was utilized for online analysis of gene expression data. BEST is a user-friendly web tool designed for clinicians and biologists to comprehensively and systematically explore the clinical significance and biological functions of cancer biomarkers without requiring bioinformatics data mining experience. Through the BEST platform, an in-depth analysis of METTL3 and METTL14 expression levels was conducted (https://rookieutopia.hiplot.com.cn/app_direct/BEST/) [19].

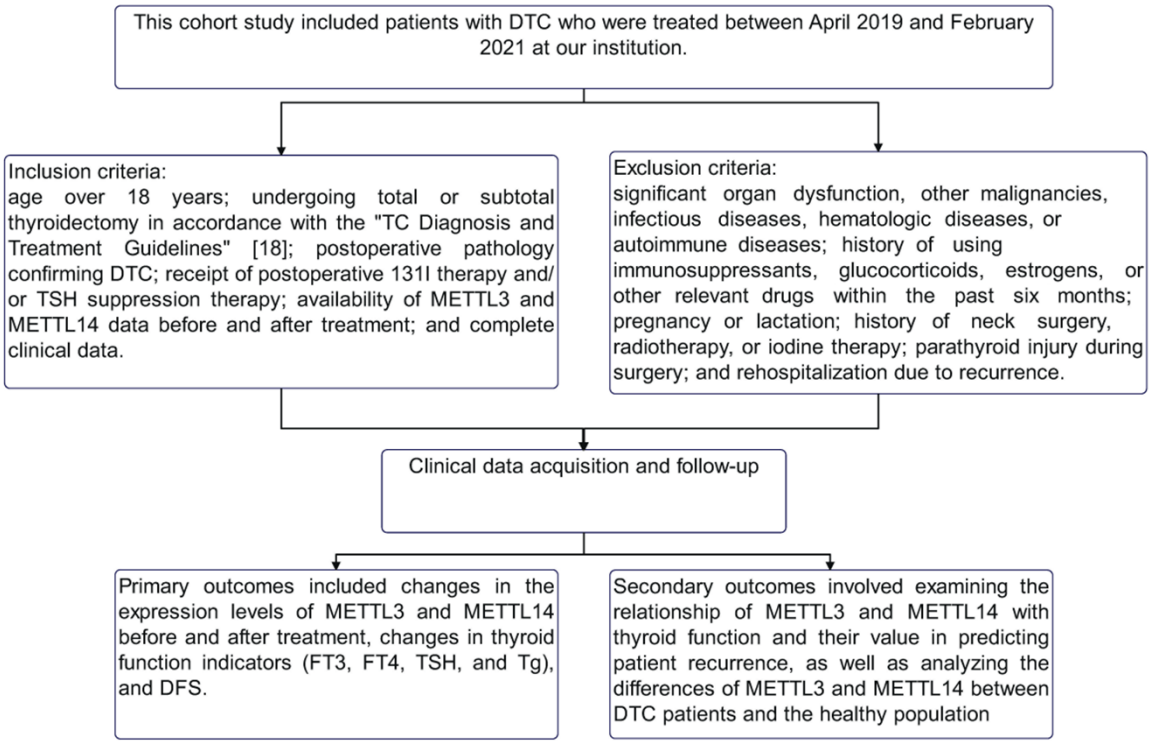


Figure 1. Study flow chart. Note: DTC, Differentiated Thyroid Cancer; TSH, Thyroid-Stimulating Hormone; Tg, Thyroglobulin; FT3, Free Triiodothyronine; FT4, Free Thyroxine; DFS, Disease-Free Survival; METTL3, Methyltransferase-Like Protein 3; METTL14, Methyltransferase-Like Protein 14.

Follow-up

Following initial surgery, patients were confirmed to have no residual tumors through imaging or serological tests. During follow-up, any new abnormalities detected by imaging, fine-needle aspiration, or serological tests were recorded as signs of recurrence. Patients were followed up every six months, with assessments including thyroid function testing, imaging examinations, and documentation of recurrence. Data were collected through telephone or outpatient follow-ups, and patients' recurrence status and survival status were meticulously recorded. Disease-free survival (DFS) refers to the period from the date of surgery until the date of either the first documented recurrence of the disease or the last follow-up without evidence of recurrence.

Outcome measures

Primary outcomes included changes in the expression levels of METTL3 and METTL14 before and after treatment, changes in thyroid function indicators (FT3, FT4, TSH, and Tg), and DFS. Secondary outcomes involved examining the relationship of METTL3 and METTL14 with thyroid function and their value in predicting patient recurrence, as well as analyzing the differences of METTL3 and METTL14 between DTC patients and the healthy population (**Figure 1**).

Statistical analysis

Data analysis was performed using SPSS 26.0 software. Initially, the distribution of all measurement data was verified using the Kolmogorov-Smirnov (KS) test. For normally distributed data, results were expressed as mean ± standard deviation, and independent sample t-tests were used for between-group comparisons, while paired t-tests were used for within-group comparisons. For data not conforming to a normal distribution, results were expressed as median and interquartile range, with the Mann-Whitney U test used for between-group comparisons and the Wilcoxon signed-rank test for within-group comparisons. Count data were analyzed using the chi-square test. Pearson correlation analysis was employed to assess

Table 1. Baseline characteristics of patients

Variable	Total	Experimental Group (n = 119)	Control Group (n = 70)	χ^2/Z Value	*P* Value
Age (years)	59.00 (56.00, 63.00)	59.00 (56.50, 62.00)	60.00 (54.25, 64.00)	-0.212	0.833
Gender				0.362	0.547
Male	59	39	20		
Female	130	80	50		
BMI (kg/m^2)	22.10 (21.00, 23.60)	22.00 (21.00, 23.60)	22.55 (21.02, 23.67)	-0.416	0.678
Clinical stage				0.397	0.529
I+II	73	48	25		
III+IV	116	71	45		
T stage				0.005	0.946
T1+T2	87	55	32		
T3+T4	102	64	38		
N stage				0.025	0.875
N0	85	53	32		
N1	104	66	38		
M stage				1.668	0.197
M0	162	105	57		
M1	27	14	13		
Tumor diameter (cm)	4.50 (3.20, 5.30)	4.50 (2.75, 5.20)	4.50 (3.70, 5.30)	-0.37	0.712
Disease type				0.262	0.609
Papillary	153	95	58		
Follicular	36	24	12		
Surgical method				1.171	0.279
Total resection	140	85	55		
Subtotal resection	49	34	15		
Number of lesions				0.057	0.811
Single	75	48	27		
Multiple	114	71	43		

Note: BMI, Body mass index.

the correlation between variables. Additionally, Cox regression analysis was performed to evaluate independent factors affecting DFS. All statistical tests were two-sided, with $P < 0.05$ considered statistically significant. Data visualization was conducted using the ggplot2 package in R to graphically present study results, including gene expression changes, distribution and trends of thyroid function indicators, and DFS survival curves.

Results

Baseline data

Comparing the baseline data between the two groups, there were no statistically significant differences in age, gender, BMI, clinical stage (I+II vs. III+IV), T stage (T1+T2 vs. T3+T4), N stage (N0 vs. N1), M stage (M0 vs. M1), tumor diameter, disease type (papillary vs. follicular), surgical method (total vs. subtotal resection), and number of lesions (single vs. multiple) ($P > 0.05$, **Table 1**).

Relative expression levels of METTL3 and METTL14 in serum of patients and healthy individuals

No statistically significant differences were found when comparing the baseline data between the patient group and the healthy population ($P > 0.05$, Table S1). Analysis of METTL3 and METTL14 expression levels in The Cancer Genome Atlas (TCGA) database using the BEST platform revealed that METTL3 and METTL14 were significantly underexpressed in the tissues of TC patients compared to adjacent non-

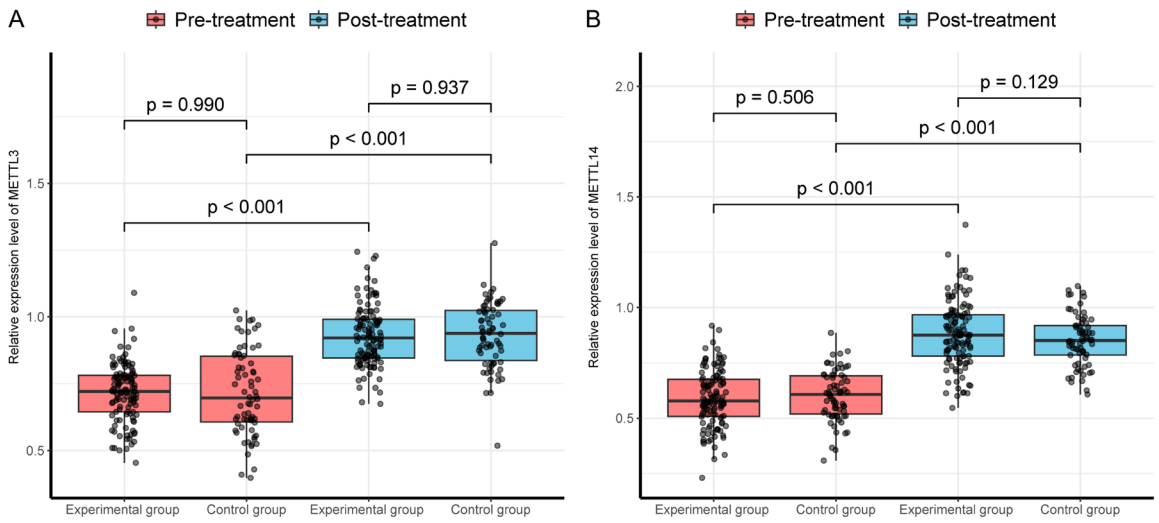


Figure 2. Changes in relative expression levels of METTL3 and METTL14 before and after treatment. A: Relative expression levels of METTL3 in the experimental and control groups before and after treatment. B: Relative expression levels of METTL14 in the experimental and control groups before and after treatment. Note: METTL, Methyltransferase-like.

tumorous tissues (P < 0.0001, Figure S1A). Further examination of METTL3 and METTL14 expression levels in the blood of 189 patients before treatment and 25 healthy individuals indicated that these genes were significantly underexpressed in the blood of DTC patients compared to those in the healthy population (P < 0.0001, Figure S1B).

Changes in relative expression levels of METTL3 and METTL14 before and after treatment

The relative expression levels of METTL3 and METTL14 were compared between the experimental and control groups before and after treatment. The results showed that the relative expression levels of METTL3 and METTL14 significantly increased after treatment in both the experimental and control groups (both P < 0.001). However, there were no significant differences in the increases between the experimental and control groups (P > 0.05, **Figure 2**).

Changes in relative expression levels of thyroid markers before and after treatment

The relative expression levels of FT3, FT4, TSH, and Tg were compared between the experimental and control groups before and after treatment. The results showed that the relative expression levels of FT3 and FT4 significantly increased after treatment in both the experimental and control groups (P < 0.001). Conversely, significant reductions were observed in the relative expression levels of TSH and Tg after treatment in both groups (P < 0.001). However, there were no significant differences in the of changes between the experimental and control groups (P > 0.05, **Figure 3**).

Correlation analysis between pre-treatment METTL3, METTL14, and thyroid markers

Correlation analysis of pre-treatment METTL3 and METTL14 with thyroid markers showed a significant positive correlation of the pre-treatment level of METTL3 with FT4 (r = 0.152, P = 0.037) and TSH (r = 0.147, P = 0.043), but no significant correlation with FT3 and Tg. Additionally, the relative expression level of METTL14 showed no significant correlation with thyroid function indicators (P > 0.05, **Figure 4**).

Correlation analysis between post-treatment METTL3, METTL14, and thyroid markers

The post-treatment expression level of METTL3 showed a significant negative correlation with TSH (r = -0.451, P < 0.001) and Tg (r = -0.388, P < 0.001), but no significant correlation with FT3 and FT4. Post-treatment, the relative expression level of METTL14 also showed a significant negative correlation with TSH (r = -0.270, P < 0.001) and Tg (r = -0.215, P = 0.003), but no significant correlation with FT3 and FT4 (**Figure 5**).

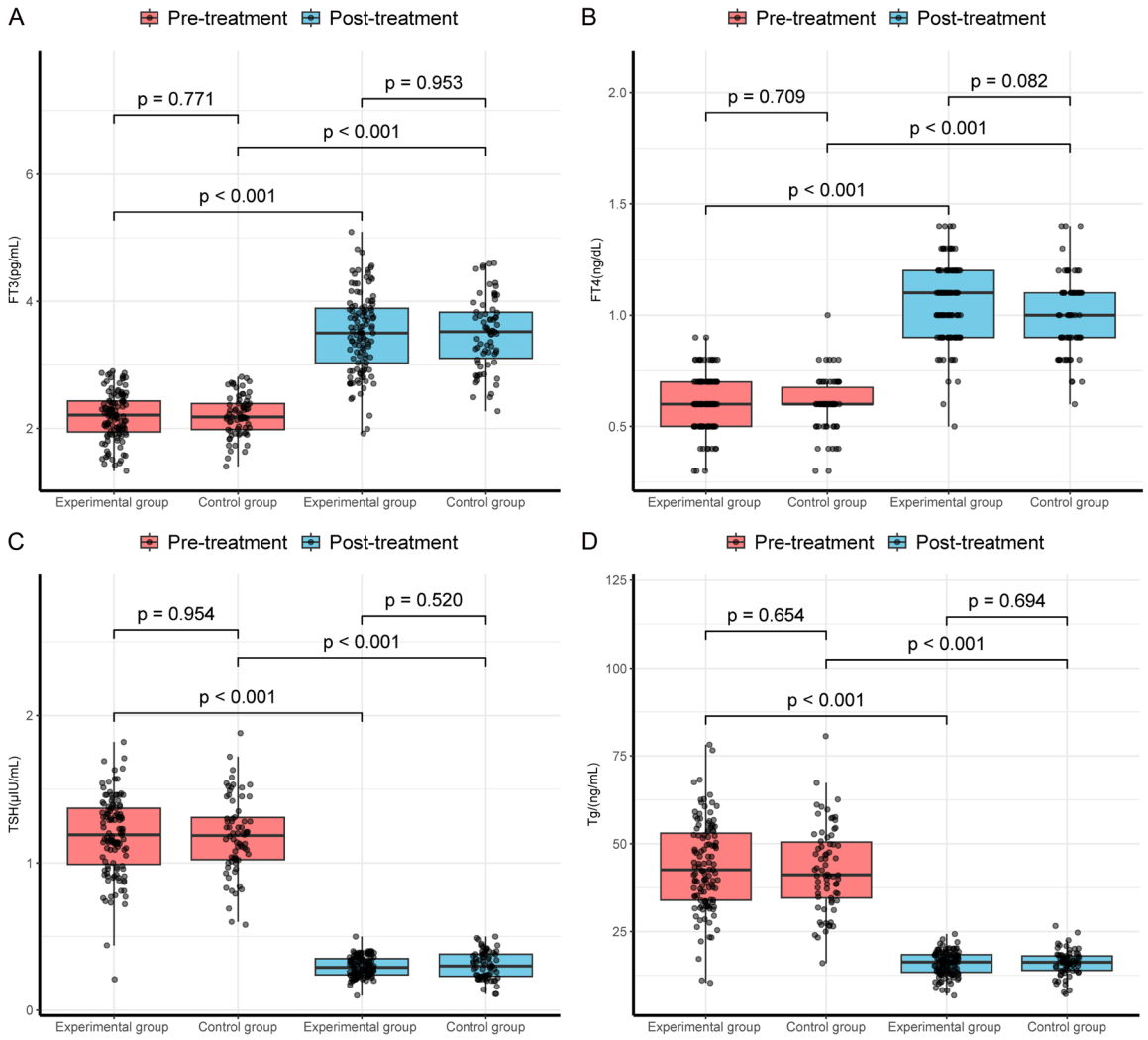


Figure 3. Changes in relative expression levels of thyroid markers (FT3, FT4, TSH, and Tg) before and after treatment. A: Relative expression levels of FT3. B: Relative expression levels of FT4. C: Relative expression levels of TSH. D: Relative expression levels of Tg. Note: FT3, Free Triiodothyronine; FT4, Free Thyroxine; TSH, Thyroid-Stimulating Hormone; Tg, Thyroglobulin.

Clinical value of METTL3, METTL14, and thyroid markers in predicting patient recurrence

The recurrence status of patients was followed for three years, with 32 out of 189 patients experiencing recurrence, with a recurrence rate of 16.9%. Patients were divided into a recurrence group (n = 32) and a non-recurrence group (n = 157) based on recurrence status. The levels of METTL3, METTL14, and thyroid markers before and after treatment were compared between the groups. The results showed that post-treatment levels of METTL3 and METTL14 were significantly lower in the recurrence group compared to the non-recurrence group (both P < 0.001). Additionally, pre-treatment METTL14 levels were significantly higher in the recurrence group than in the non-recurrence group (P = 0.045). Regarding thyroid markers, post-treatment levels of TSH and Tg were significantly higher in the recurrence group compared to those in the non-recurrence group (both P < 0.001). No significant differences were found in the remaining indicators between the two groups (P > 0.05, **Table 2**; **Figure 6**).

Cox regression analysis of prognostic factors affecting DFS

In the Cox regression analysis, several factors were included in the univariate analysis based

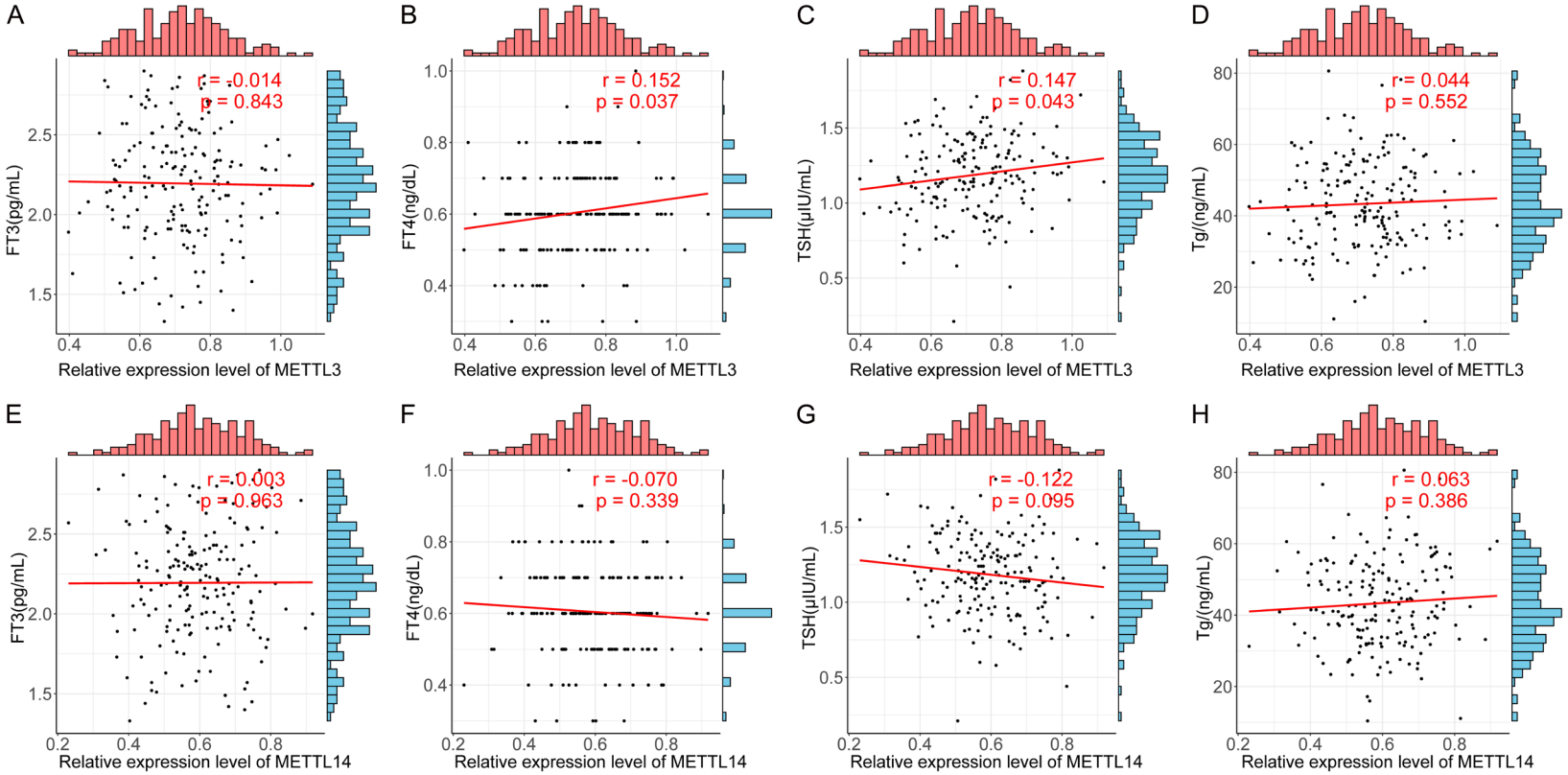


Figure 4. Correlation analysis between pre-treatment METTL3, METTL14, and thyroid function indicators. (A-D) Scatter plots showing the correlation between pre-treatment METTL3 levels and thyroid function indicators: (A) FT3, (B) FT4, (C) TSH, and (D) Tg. (E-H) Scatter plots showing the correlation between pre-treatment METTL14 levels and thyroid function indicators: (E) FT3, (F) FT4, (G) TSH, and (H) Tg. Significant correlations were observed between METTL3 and FT4, METTL3 and TSH, METTL14 and FT3, and METTL14 and FT4. Note: FT3, Free Triiodothyronine; FT4, Free Thyroxine; TSH, Thyroid-Stimulating Hormone; Tg, Thyroglobulin; METTL3, Methyltransferase-like 3; METTL14, Methyltransferase-like 14.

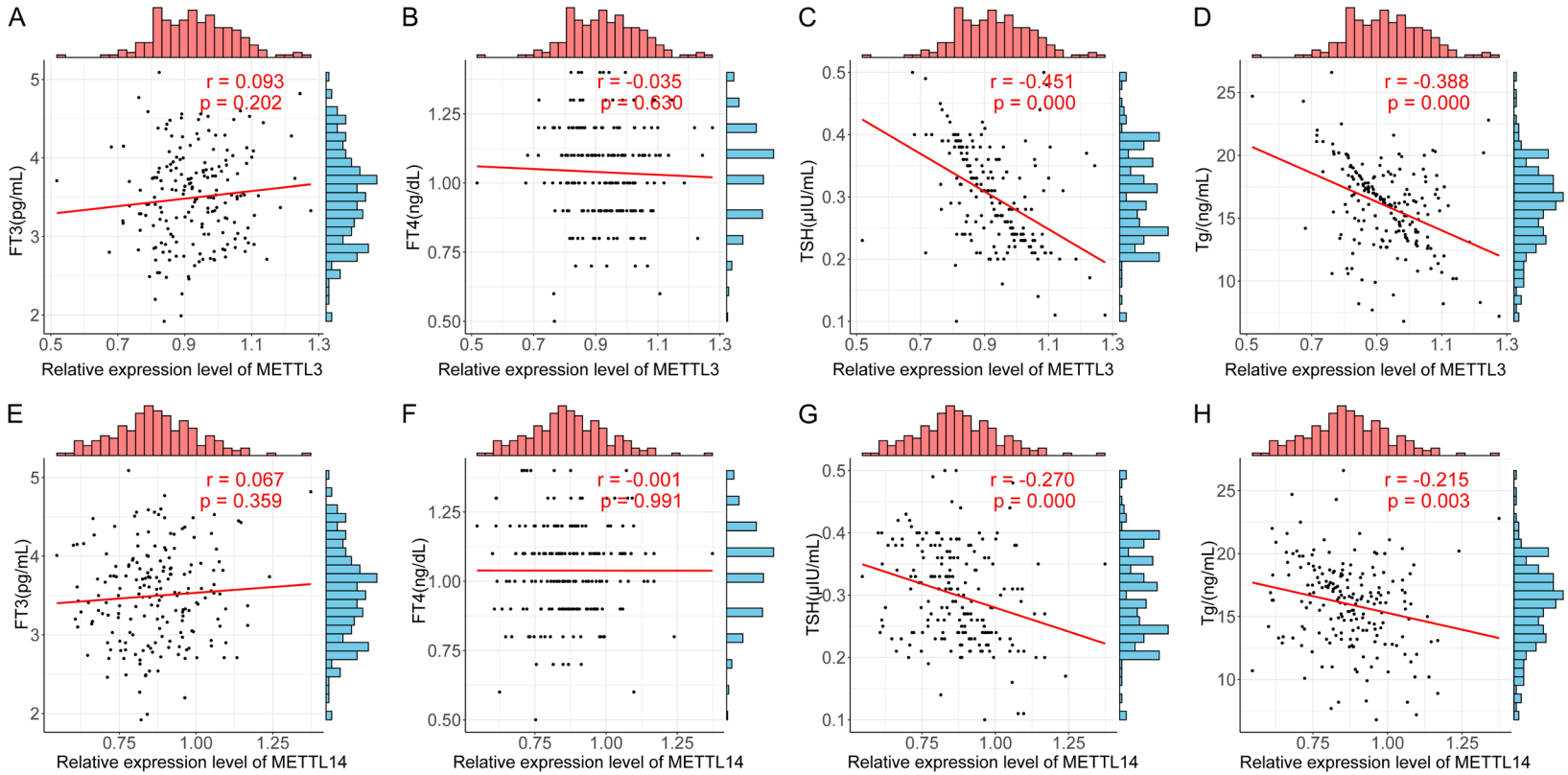


Figure 5. Correlation analysis between post-treatment METTL3, METTL14, and thyroid function indicators. (A-D) Scatter plots showing the correlation between post-treatment METTL3 levels and thyroid function indicators: (A) FT3, (B) FT4, (C) TSH, and (D) Tg. (E-H) Scatter plots showing the correlation between post-treatment METTL14 levels and thyroid function indicators: (E) FT3, (F) FT4, (G) TSH, and (H) Tg. Significant correlations were observed between METTL3 and TSH, METTL3 and Tg, METTL14 and TSH, and METTL14 and Tg. Note: FT3, Free Triiodothyronine; FT4, Free Thyroxine; TSH, Thyroid-Stimulating Hormone; Tg, Thyroglobulin; METTL3, Methyltransferase-like 3; METTL14, Methyltransferase-like 14.

Table 2. Levels of METTL3, METTL14, and thyroid markers in recurrent patients

Variable	Recurrence Group (n = 32)	Non-recurrence Group (n = 157)	t/Z Value	*P* Value
Pre-treatment METTL3	0.68 ± 0.16	0.72 ± 0.12	-1.295	0.203
Post-treatment METTL3	0.81 [0.77, 0.88]	0.95 ± 0.10	-6.598	< 0.001
Pre-treatment METTL14	0.62 ± 0.11	0.59 ± 0.12	1.3	0.2
Post-treatment METTL14	0.77 ± 0.09	0.89 ± 0.14	-5.972	< 0.001
Pre-treatment FT3 (pg/mL)	2.23 ± 0.33	2.19 ± 0.36	0.596	0.554
Post-treatment FT3 (pg/mL)	3.46 ± 0.52	3.51 ± 0.61	-0.455	0.651
Pre-treatment FT4 (ng/dL)	0.60 [0.50, 0.70]	0.60 [0.50, 0.70]	-0.479	0.618
Post-treatment FT4 (ng/dL)	1.10 [0.90, 1.10]	1.00 [0.90, 1.20]	-0.406	0.681
Pre-treatment TSH (μIU/mL)	1.20 ± 0.23	1.18 ± 0.27	0.404	0.688
Post-treatment TSH (μIU/mL)	0.38 [0.33, 0.40]	0.28 [0.24, 0.34]	4.716	< 0.001
Pre-treatment Tg (ng/mL)	44.29 ± 10.79	43.17 ± 12.63	0.52	0.605
Post-treatment Tg (ng/mL)	18.41 ± 3.43	16.00 [13.40, 17.70]	4.116	< 0.001

Note: METTL, Methyltransferase-like; FT3, Free Triiodothyronine; FT4, Free Thyroxine; TSH, Thyroid-Stimulating Hormone; Tg, Thyroglobulin; METTL3, Methyltransferase-like 3; METTL14, Methyltransferase-like 14.

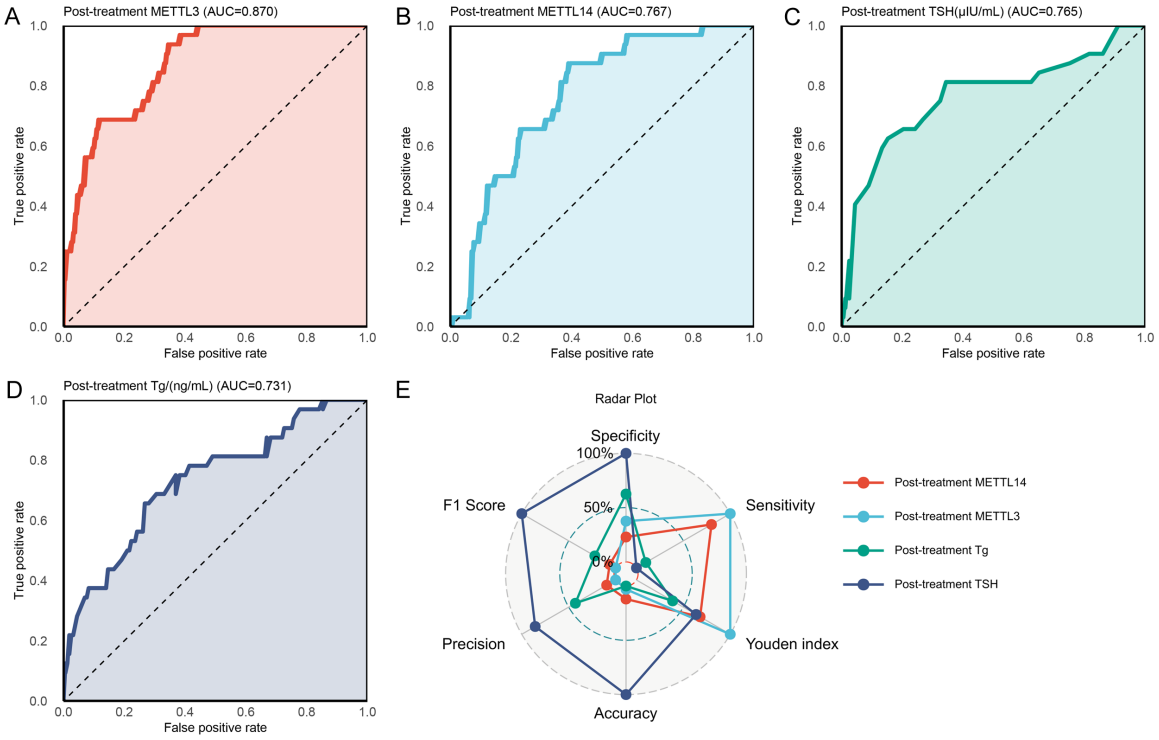


Figure 6. ROC curves of significant markers for predicting recurrence in patients. A: ROC curve for post-treatment METTL3 in predicting recurrence. B: ROC curve for post-treatment METTL14 in predicting recurrence. C: ROC curve for post-treatment TSH in predicting recurrence. D: ROC curve for post-treatment Tg in predicting recurrence. E: Radar plot displaying the ROC parameters (AUC and Cut-off values) for all significant markers. Note: METTL3, Methyltransferase-like 3; METTL14, Methyltransferase-like 14; TSH, Thyroid-Stimulating Hormone; Tg, Thyroglobulin; AUC, Area Under the Curve.

on their potential relevance to DFS. These factors included: treatment regimen (P < 0.001), clinical stage (P < 0.001), N stage (P = 0.001), surgical method (P = 0.039), number of lesions (P = 0.005), pre-treatment METTL3 (P = 0.017), post-treatment METTL3 (P < 0.001), post-treatment METTL14 (P < 0.001), post-treatment TSH (P < 0.001), as well as pre-treatment Tg (P

Table 3. Univariate cox regression analysis

Factor	β	SE	*P* Value	HR	Lower	Upper
Treatment Program	-1.637	0.394	< 0.001	0.195	0.09	0.421
Age (years)	1.409	0.487	0.004	4.093	1.576	10.631
Gender	0.314	0.365	0.39	1.369	0.669	2.801
BMI (kg/m^2)	0.608	0.428	0.155	1.836	0.794	4.247
Clinical stage	1.323	0.269	< 0.001	3.755	2.217	6.36
T stage	0.313	0.183	0.088	1.367	0.955	1.958
N stage	1.59	0.487	0.001	4.903	1.888	12.734
M stage	-1.73	1.016	0.089	0.177	0.024	1.299
Tumor diameter (cm)	0.81	0.487	0.096	2.249	0.866	5.84
Disease type	-0.003	0.453	0.995	0.997	0.41	2.423
Surgical method	0.741	0.36	0.039	2.099	1.036	4.251
Number of lesions	1.354	0.487	0.005	3.873	1.491	10.06
Pre-treatment METTL3	-0.849	0.354	0.017	0.428	0.214	0.857
Post-treatment METTL3	-2.303	0.374	< 0.001	0.1	0.048	0.208
Pre-treatment METTL14	0.664	0.372	0.075	1.942	0.936	4.028
Post-treatment METTL14	-1.941	0.487	< 0.001	0.144	0.055	0.373
Pre-treatment FT3 (pg/mL)	0.633	0.607	0.297	1.883	0.574	6.182
Post-treatment FT3 (pg/mL)	-0.667	0.428	0.119	0.513	0.222	1.187
Pre-treatment FT4 (ng/dL)	-0.402	0.535	0.452	0.669	0.235	1.907
Post-treatment FT4 (ng/dL)	-1.02	0.607	0.093	0.36	0.11	1.184
Pre-treatment TSH (μIU/mL)	1.238	0.73	0.09	3.449	0.824	14.433
Post-treatment TSH (μIU/mL)	1.88	0.356	< 0.001	6.553	3.264	13.159
Pre-treatment Tg (ng/mL)	0.996	0.428	0.02	2.708	1.171	6.261
Post-treatment Tg (ng/mL)	1.484	0.366	< 0.001	4.412	2.154	9.037

Note: BMI, Body Mass Index; T, Tumor stage; N, Node stage; M, Metastasis stage; METTL3, Methyltransferase-like 3; METTL14, Methyltransferase-like 14; FT3, Free Triiodothyronine; FT4, Free Thyroxine; TSH, Thyroid-Stimulating Hormone; Tg, Thyroglobulin.

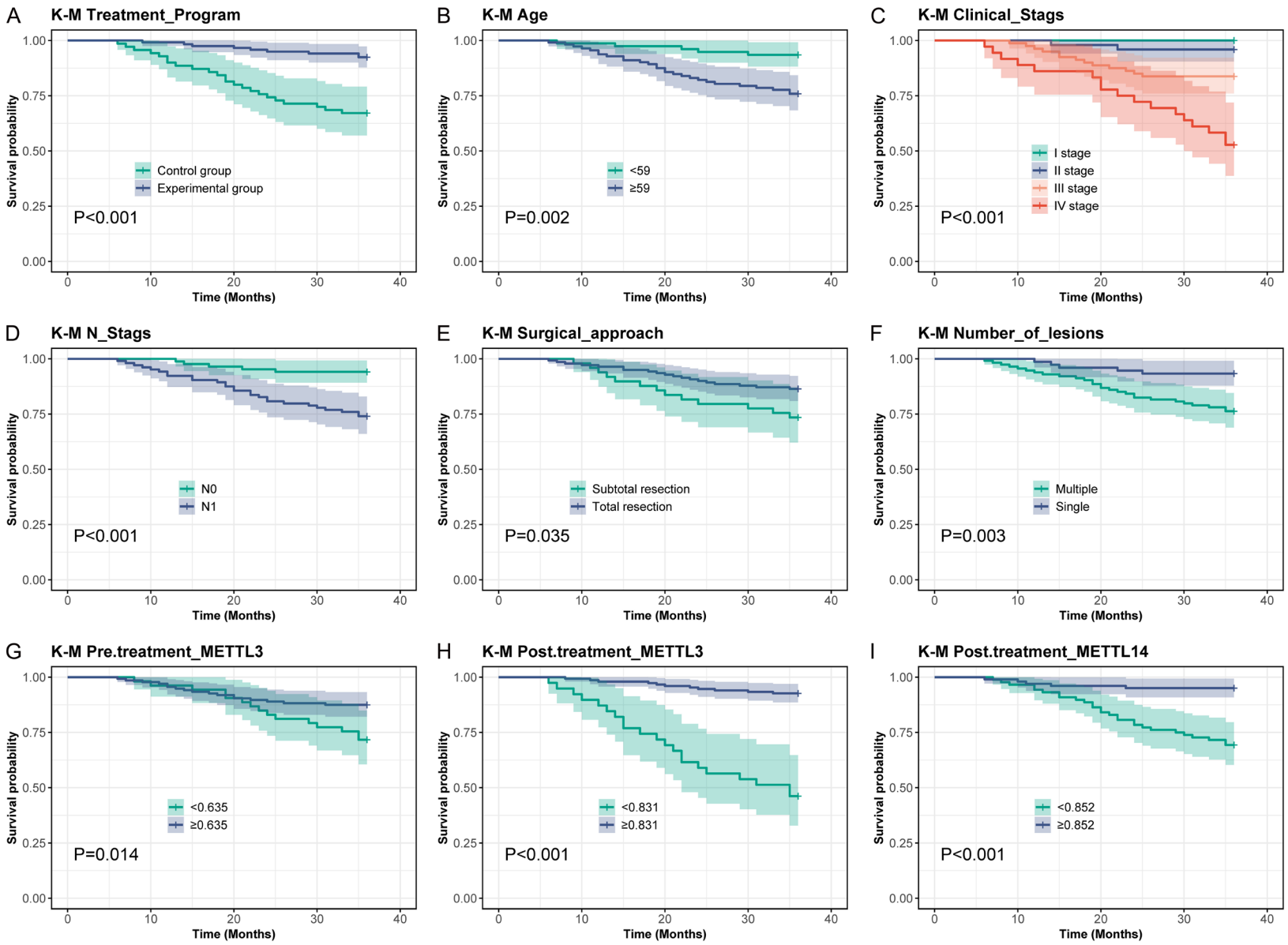
= 0.020) and post-treatment Tg (P < 0.001) (**Table 3**; **Figure 7**). These factors were selected based on their clinical significance and findings from prior literature indicating their potential impact on DFS. METTL3, METTL14, TSH, and Tg were included due to their known roles in tumor biology. The results showed that post-treatment METTL3 (hazard ratio [HR] = 0.100, P < 0.001), post-treatment METTL14 (HR = 0.144, P < 0.001), post-treatment TSH (HR = 6.553, P < 0.001), and post-treatment Tg (HR = 4.412, P < 0.001) had significant prognostic value.

Multivariate cox regression analysis

Multivariate Cox regression analysis further identified independent prognostic factors, including treatment regimen (HR = 0.057, P < 0.001), N stage (HR = 19.353, P < 0.001), surgical method (HR = 2.887, P = 0.016), post-treatment METTL3 (HR = 0.107, P < 0.001), post-treatment METTL14 (HR = 0.254, P = 0.030), and post-treatment TSH (HR = 7.707, P < 0.001) (**Table 4**). These factors were selected for multivariate analysis based on their statistical significance (P < 0.05) in the univariate analysis and their clinical relevance. The multivariate analysis aimed to control for confounding factors, identifying these as independent predictors of DFS.

Discussion

This study is the first to systematically evaluate changes in the expression levels of METTL3 and METTL14 in patients with DTC and their relationship with thyroid function indicators. The results demonstrate that METTL3 and METTL14 expression levels significantly decreased after treatment, with low expression levels significantly associated with an increased risk of recurrence. Notably, patients receiving combined radioactive iodine (^{131}I) and TSH sup-


A
K-M Treatment_Program
Survival probability
Time (Months)
Control group
Experimental group
P<0.001
B
K-M Age
<59
≥59
P=0.002
C
K-M Clinical_Stags
I stage
II stage
III stage
IV stage
P<0.001
D
K-M N_Stags
N0
N1
P<0.001
E
K-M Surgical_approach
Subtotal resection
Total resection
P=0.035
F
K-M Number_of_lesions
Multiple
Single
P=0.003
G
K-M Pre.treatment_METTL3
<0.635
≥0.635
P=0.014
H
K-M Post.treatment_METTL3
<0.831
≥0.831
P<0.001
I
K-M Post.treatment_METTL14
<0.852
≥0.852
P<0.001

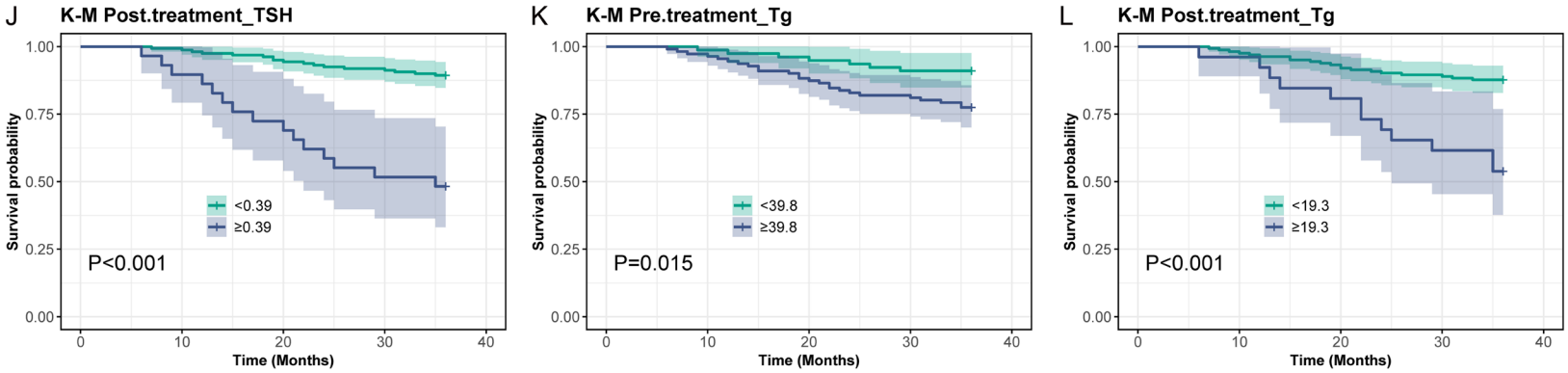


Figure 7. Kaplan-Meier curves for DFS based on significant factors identified in univariate cox regression analysis. Survival probabilities are shown for key factors: (A) Treatment program, (B) Age, (C) Clinical stage, (D) N stage, (E) Surgical approach, (F) Number of lesions, (G) Pre-treatment METTL3, (H) Post-treatment METTL3, (I) Post-treatment METTL14, (J) Post-treatment TSH, (K) Pre-treatment Tg, and (L) Post-treatment Tg. Note: DFS, Disease-Free Survival; TSH, Thyroid-Stimulating Hormone; Tg, Thyroglobulin; METTL3, Methyltransferase-like 3; METTL14, Methyltransferase-like 14.

Table 4. Multivariate cox regression analysis

Factor	Beta	SE	*P* Value	HR	Lower	Upper
Treatment Program	-2.861	0.568	< 0.001	0.057	0.019	0.174
Age (years)	0.111	0.589	0.85	1.118	0.353	3.544
Clinical stage	-0.04	0.463	0.932	0.961	0.388	2.383
N stage	2.963	0.788	< 0.001	19.353	4.134	90.594
Surgical method	1.06	0.439	0.016	2.887	1.22	6.831
Number of lesions	1.034	0.744	0.165	2.813	0.654	12.096
Pre-treatment METTL3	-0.32	0.413	0.438	0.726	0.323	1.631
Post-treatment METTL3	-2.237	0.601	< 0.001	0.107	0.033	0.347
Post-treatment METTL14	-1.37	0.632	0.03	0.254	0.074	0.877
Post-treatment TSH (μIU/mL)	2.042	0.504	< 0.001	7.707	2.869	20.698
Pre-treatment Tg (ng/mL)	0.987	0.531	0.063	2.682	0.948	7.589
Post-treatment Tg (ng/mL)	-0.126	0.506	0.803	0.882	0.327	2.377

Note: METTL3, Methyltransferase-like 3; METTL14, Methyltransferase-like 14; TSH, Thyroid-Stimulating Hormone; Tg, Thyroglobulin.

pression therapy exhibited longer DFS, suggesting that METTL3 and METTL14 may serve as important markers for prognosis and personalized treatment in DTC patients.

To explore the underlying mechanisms behind these observations, we analyzed the impact of ^{131}I and TSH suppression therapy on METTL3 and METTL14 levels. The decrease in METTL3 and METTL14 expression post-treatment may be due to ^{131}I therapy's targeted destruction of thyroid cells. ^{131}I specifically destroys residual thyroid tissue and cancer cells by radioactive iodine uptake, thereby reducing the number of thyroid cells expressing METTL3 and METTL14. Wu et al. [20] found that METTL3 overexpression in various cancers correlates with tumor progression, with its expression significant decreased post-treatment. Similarly, Jiang et al. [21] reported that high METTL3 expression in hepatocellular carcinoma patients could predict poor prognosis, while decreased expression correlated with treatment efficacy. Furthermore, TSH suppression therapy reduces TSH secretion, inhibiting the proliferation and activity of thyroid cells, which may contribute to the observed decrease in METTL3 and METTL14 expression.

The observed correlation between post-treatment METTL3 and METTL14 levels and thyroid function indicators, such as TSH and Tg, may reflect an indirect regulatory role of these genes in thyroid function. TSH is the main regulator of thyroid hormone synthesis, while Tg, as a precursor of thyroid hormones, is essential in maintaining thyroid function [22]. Zhu et al. [23] demonstrated that METTL3 regulates gene expression through m6A modification, potentially affecting genes critical to thyroid function, such as TSH and Tg. Additionally, Zhou et al. [24] reported that METTL3 influences cancer cell proliferation and metastasis via interactions with specific lncRNAs, which may similarly impact DTC patients post-treatment. These findings suggest that METTL3 and METTL14 may indirectly contribute to changes in thyroid function observed in DTC patients after treatment.

DTC recurrence poses a substantial risk to long-term patient outcomes and quality of life [25]. Recurrence often signifies resistance to initial treatments or the growth of residual lesions, necessitating more complex therapies, including re-surgery, radioactive iodine, and even chemotherapy, which can lead to increased treatment risks and decreased survival rates [26, 27]. Therefore, accurately predicting recurrence risk and implementing individualized treatment strategies are essential for improving patient prognosis.

In this study, we compared METTL3 and METTL14 levels before and after treatment in patients with and without recurrence and analyzed their relationship with thyroid function indicators. Our findings revealed significantly lower post-treatment levels of METTL3 and METTL14 in the recurrence group compared to

those in the non-recurrence group, suggesting that low METTL3 and METTL14 expression may be associated with an increased recurrence risk. Furthermore, pre-treatment METTL14 levels were notably higher in the recurrence group, indicating a potential role in early tumor progression. Additionally, the recurrence group displayed significantly higher post-treatment TSH and Tg levels, suggesting that thyroid function changes may play a critical role in the recurrence process. Elevated TSH and Tg levels may indicate increased thyroid cell sensitivity to TSH or heightened activity in residual thyroid tissue or cancer cells [22, 28]. Low METTL3 and METTL14 levels may reduce mRNA stability of tumor suppressor genes in TC cells, promoting malignant progression and tumor recurrence. Through gene expression regulation and mRNA stability, METTL3 and METTL14 may indirectly influence the recurrence risk in DTC patients [29, 30]. These findings offer new directions for further investigation into the prognostic potential of METTL3 and METTL14 in DTC.

To understand the long-term impact of METTL3 and METTL14 expression on DTC prognosis, we analyzed 3-year DFS rates in this patient population. Cox regression analysis showed that low post-treatment expression of METTL3 and METTL14 was significantly associated with a higher recurrence risk, underscoring their predictive value in long-term prognosis. Zhang et al. [31] reported similar findings in TC patients, where low METTL3 expression correlated with poor prognosis, consistent with our study. These results suggest that METTL3 and METTL14 may influence tumor recurrence by stabilizing mRNAs of tumor suppressor genes, thereby regulating key signaling pathways involved in tumorigenesis.

Further analysis of DFS identified additional factors impacting DTC prognosis. Notably, patients receiving combined ^{131}I and TSH suppression therapy had a lower recurrence risk than those receiving TSH suppression alone, likely due to combined therapy's ability to clear residual thyroid tissue and cancer cells more effectively. Ye et al. [16] reported associations between tumor size, multiplicity, and the number of neck lymph node metastases with DTC recurrence. Elevated TSH levels also emerged as a significant predictor of recurrence. Lee et al. [32] demonstrated that TSH suppression therapy reduced recurrence risk in intermediate- and low-risk TC patients. Although Gubbi et al. [33] indicated that TSH suppression may not improve survival in high-risk patients, it may reduce recurrence risk, though with increased cardiovascular and bone metabolic complications. Additionally, Watanabe et al. [34] found that post-treatment Tg levels closely correlated with DFS, with elevated Tg suggesting a higher recurrence risk, consistent with our finding of higher TSH and Tg levels in the recurrence group.

Conclusion

This study systematically evaluated METTL3 and METTL14 expression changes and their relationship with thyroid function indicators in DTC patients. We found that combined ^{131}I and TSH suppression therapy significantly reduced METTL3 and METTL14 expression, and low expression levels were associated with an increased recurrence risk. However, this study has several limitations. The small sample size may not fully capture the effects of various treatments. As a retrospective study, it is subject to selection and information bias, limiting causal inference. The follow-up period is relatively short, spanning only three years, which may miss late recurrences, and the lack of external validation restricts the generalizability of our results. Future research should consider larger sample sizes, extended follow-up, prospective design, and external validation to further clarify the prognostic roles of METTL3 and METTL14 in DTC.

Disclosure of conflict of interest

None.

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Table S1. Baseline data of patient group and normal population

Variable	Total	Patient Group (n = 189)	Normal person (n = 25)	χ^2/Z Value	*P* Value
Age (years)		59.00 (56.00, 63.00)	60.00 (57.00, 62.00)	-0.805	0.421
Gender					
Male		59	9	0.233	0.629
Female		130	16		
BMI (kg/m^2)		22.10 (21.00, 23.60)	22.18 ± 1.14	0.433	0.666

Note: Body mass index (BMI).

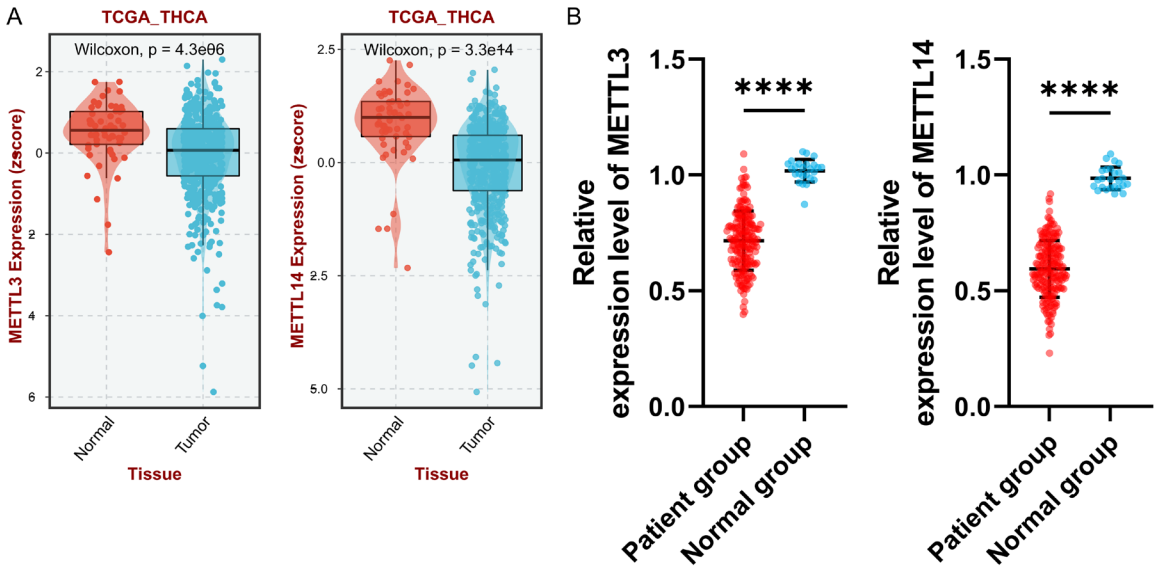

Figure S1. METTL3 and METTL14 expression levels in thyroid cancer tissue and serum. A. METTL3 and METTL14 expression levels in thyroid cancer tissues and adjacent normal tissues from the TCGA database, Both were significantly downregulated in cancer tissues (P < 0.0001). B. Relative expression levels of METTL3 and METTL14 in the serum of thyroid cancer patients and healthy individuals, Both were significantly lower in patients (P < 0.0001). Note: The Cancer Genome Atlas (TCGA), Biomarker Exploration and Signature Test (BEST), and Methyltransferase-like (METTL); ****P < 0.0001 indicates a statistically significant difference.