Exploring the Role of Non-Coding RNAs in Papillary Thyroid Carcinoma Progression: A qPCR-Based Approach

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Abstract

Background: Papillary thyroid carcinoma (PTC) is the predominant kind of thyroid cancer, with certain patients exhibiting an aggressive disease course marked by recurrence and metastasis. Non-coding RNAs (ncRNAs) have recently been acknowledged for their involvement in cancer progression, indicating their potential as prognostic indicators. This study examines the expression of specific ncRNAs, namely miR-146b, H19, and MALAT1, in PTC. **Objectives:** The project seeks to characterise the expression of miR-146b, H19, and MALAT1 in papillary thyroid carcinoma (PTC) compared to normal thyroid tissues to evaluate their potential as prognostic biomarkers, linking their levels with tumour attributes including size, metastasis, and recurrence. **Methods:** Seventy-five individuals were categorised into three groups: normal thyroid tissue, low-risk papillary thyroid carcinoma (PTC), and high-risk papillary thyroid carcinoma (PTC). RNA was isolated from tissue samples and transcribed into complementary DNA (cDNA) for quantitative PCR (qPCR). The expression levels of the target ncRNAs were standardised to GAPDH, and statistical analyses were performed to compare the groups. **Results:** qPCR analysis revealed markedly increased expression of miR-146b, H19, and MALAT1 in both PTC groups compared to the normal group, with the highest expression noted in high-risk PTC. ΔCt values demonstrated considerable disparities, especially for MALAT1, indicating its potential as a biomarker for PTC aggressiveness. **Conclusion:** The results endorse miR-146b, H19, and MALAT1 as potential biomarkers for differentiating PTC from normal thyroid tissue. MALAT1's expression profile correlates with tumour aggressiveness, highlighting its potential as a prognostic instrument for PTC therapy.

Keywords: Papillary Thyroid Carcinoma (PTC), Non-coding RNAs, Biomarkers, qPCR, MALATI.

Introduction

Papillary thyroid carcinoma (PTC) is the predominant variant of thyroid cancer, with a range of clinical behaviours from indolent to extremely aggressive types. A portion of PTC patients, despite the usually favourable prognosis, encounters an elevated risk of disease recurrence and metastasis, presenting a considerable clinical care challenge. [1] At now, conventional histological procedures and imaging techniques do not consistently forecast which individuals would undergo aggressive disease development. Consequently, there is an urgent necessity for biomarkers that facilitate the early identification of high-risk patients, thereby guiding more tailored treatment approaches.[2]

The quest for effective biomarkers in PTC centres on identifying molecular indicators that correlate with tumour aggressiveness and metastatic capability. Genomic changes, such as BRAF V600E mutations, are linked to increased tumour invasiveness and recurrence, highlighting their prognostic significance.[3] Recent studies have emphasised the significance of inflammatory biomarkers and non-coding RNAs in forecasting central lymph node metastases and disease aggressiveness, hence broadening the array of possible biomarkers for risk evaluation in PTC.[4]

Advancements in biomarker research promise to enhance our comprehension of PTC progression and improve predictive precision. The incorporation of these indicators into clinical practice has the potential to transform PTC therapy by facilitating earlier, more precise interventions and eventually enhancing patient outcomes.

This study aimed to examine the expression patterns of particular non-coding RNAs, including microRNAs and long non-coding RNAs, in papillary thyroid cancer. The

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objective was to clarify their potential involvement in the advancement of PTC by analysing their expression in cancer tissues relative to corresponding normal thyroid tissues. This work aimed to investigate the correlation between ncRNA expression levels and significant clinical characteristics, including tumour size, metastasis, and recurrence, to evaluate the potential of these ncRNAs as prognostic markers or therapeutic targets in PTC.

Methodology

Patient Recruitment and Group Categorization

We allocated 75 patients evenly into three groups of 25 patients each to examine the impact of non-coding RNAs in the evolution of papillary thyroid cancer (PTC). The groups were delineated according to health condition and cancer attributes as follows:

Normal Thyroid Group (Control Group): Patients who underwent thyroid surgery for benign conditions unrelated to the disease under investigation (e.g., hyperthyroidism, benign goitre) and whose postoperative microscopic analysis revealed no malignant or premalignant pathology.

Early-Stage Papillary Thyroid Carcinoma (PTC) with Low Tumour Characteristics: Patients exhibit papillary thyroid carcinoma characterised by a diminutive tumour size (T1 stage), absence of clinical or radiological indications of metastasis, and no documented instances of tumour recurrence.

PTC with Advanced Tumour Characteristics (Advanced-Stage PTC): Patients diagnosed with papillary thyroid cancer exhibiting increased tumour dimensions (T2 stage or above), verified metastases through radiologic and clinical assessments, and a recorded history of recurrence.

Sample Size Justification and Power Calculations

The sample size of 25 patients per group was established by statistical power analysis to guarantee adequate power for identifying significant variations in non-coding RNA expression among the groups. A minimum power of 80% (β = 0.2) was established as the criterion, with a significance level (α) of 0.05. Drawing from previous research on ncRNA expression patterns in thyroid and various malignancies, the effect magnitude for expected discrepancies in ncRNA levels between normal and malignant thyroid tissues was assessed. Based on an effect size (Cohen's d) of 0.75, the subsequent sample size estimates were performed:

1. Two-Sample t-Test for Independent Means (Normal vs. Cancer Groups): Using the formula for a two-sample t-test with an estimated effect size (d) of 0.75, power of 0.8, and α of 0.05:

n=((Zα/2+Zβ)2×(2σ2)Δ2)n = \left(\frac{(Z_{\alpha/2} + Z_{\beta})^2 \times (2\sigma^2)}{\Delta^2} \right) n=(Δ2(Zα/2+Zβ)2×(2σ2))

where Zα/2Z_{\alpha/2}Zα/2 and ZβZ_{\beta}Zβ correspond to the z-scores for a two-tailed α of 0.05 and β of 0.2, respectively, σ\sigmaσ is the standard deviation, and Δ\DeltaΔ is the mean difference. Based on preliminary data for ncRNA expression in thyroid tissue, calculations supported a minimum of 20 patients per group. To account for variability in clinical data, a conservative target of 25 patients per group was chosen.

2. Sample Size for Paired Comparisons (Early-Stage vs. Advanced-Stage PTC): To detect differences in ncRNA expression between early- and advanced-stage PTC, a similar calculation was applied with an effect size estimated at 0.7, suggesting 23 participants per group. Therefore, Robust comparative power was ensured across all groups and we recruited 25 patients per group.

3. Adjustment for Missing Data: The inclusion of a buffer of approximately 5% for missing or incomplete data in the sample size calculation helped to justify our decision that we should recruit 25 patients per group.

Ethical Considerations

The Institutional Review Board (IRB) of the associated medical institution granted ethical approval. All individuals supplied informed consent and received specific attention in the normal thyroid group who had undergone surgery for benign conditions. Participants were informed that the study focused on cancer biomarkers and that their involvement would not affect treatment outcomes or necessitate further surgical interventions. Tissue samples utilised for patients were obtained from normal surgical procedures, and no supplementary invasive procedures were required for research purposes for any patients. Patient data was maintained confidentially in accordance with the Declaration of Helsinki and institutional regulations. The study aimed to enhance comprehension of PTC progression and its possible implications for future therapeutic advancements, with patients in each cancer group informed that this research sought to further information regarding PTC progression.

Inclusion and Exclusion Criteria

Inclusion Criteria

- Adults aged 18 years or older.
- Availability of sufficient thyroid tissue from routine thyroidectomy.
- Confirmed histopathologic diagnosis of PTC for cancer groups, with subgrouping based on tumor characteristics.
- Confirmed benign pathology for the normal group with no history of thyroid cancer or pre-malignant conditions.

Exclusion Criteria

- Patients with prior or concurrent malignancies outside of thyroid cancer.
- Patients with significant autoimmune thyroid disease (e.g., Hashimoto's thyroiditis).
- Patients with comorbid conditions likely to influence ncRNA expression profiles.
- Insufficient tissue availability or incomplete clinical data.

Diagnostic and Grouping Criteria

1. **Papillary Thyroid Carcinoma Diagnosis:** Diagnosis of PTC was confirmed histologically by pathologists based

on characteristic nuclear features, including nuclear grooves, pseudoinclusions, and overlapping nuclei.

2. **Metastasis Status:** Metastasis determination was based on clinical, histologic, and radiologic evidence, with metastasis confirmed by positive findings in regional lymph nodes or distant organs.
3. **Tumor Size Classification:** Tumor size was classified according to the American Joint Committee on Cancer (AJCC) TNM staging system:
    - *T1:* Tumors ≤2 cm in greatest dimension, included in the early-stage PTC group.
    - *T2 and Higher Stages:* Tumors >2 cm, included in the advanced-stage PTC group.

Sample Collection and Preparation

The sample collection and preparation process was rigorously structured to guarantee the acquisition and handling of high-quality tissue samples, hence preserving the integrity of non-coding RNA (ncRNA) molecules. Each subject, having satisfied the inclusion criteria and provided informed consent, supplied thyroid tissue samples during a standard surgical procedure (thyroidectomy). Tissue specimens from the primary tumour location were obtained from patients in the papillary thyroid cancer (PTC) cohort, whereas samples from the thyroid gland devoid of any malignant or pre-malignant lesions were procured from patients in the normal cohort.

The tissue samples were swiftly moved to sterile, RNase-free containers that were pre-cooled to reduce RNA degradation following surgical excision. The containers with the RNA preservation solution were filled with RNA to maintain stability and assure the reliability of subsequent studies. Samples were transported to the laboratory at regulated temperatures, with specimens maintained on ice to avert any temperature variations that could compromise RNA purity. Samples were sent directly to the laboratory for prompt processing or stored at -80°C if processing was postponed. We examined each sample in the laboratory, searching for unsuitable tissue. Tissues were sectioned into fresh-frozen samples to acquire roughly 30 mg of tissue per sample by excising as much non-target tissue as feasible. To isolate representative PTC samples, meticulously selected tumour areas were utilised to ensure the sample had a homogeneous portion of cancer cells. Likewise, noncancerous tissue areas were identified for the control group.

RNA quality was meticulously maintained through the subsequent RNA extraction technique. The extracted RNA was further processed with DNase to eliminate any leftover DNA that could interfere with the quantification of non-coding RNAs, ensuring the accuracy of the measurement by removing genomic material. To evaluate the suitability of RNA molecules for further investigation, additional checks of concentration and purity were conducted using spectrophotometric methods to confirm RNA integrity. The meticulous methodology employed in this sample collection and preparation was crucial for ensuring sufficient quality for reliable qPCR-based quantification of target miRNAs and lncRNAs.

During the sample collection and preparation process, we emphasised steps that maintained molecular integrity, as the accuracy of our findings is entirely contingent upon the purity of our samples at each stage, from excision to RNA extraction.

Tissue Sample RNA Extraction

The acquisition of thyroid tissues was merely the initial phase in the extraction of high-quality RNA. Initially thawed on ice in the laboratory to prevent thermal shock that could compromise RNA integrity, each tissue sample was meticulously kept to ensure RNA stability. We meticulously managed the samples to encompass the complete range of RNA molecules, particularly the delicate yet crucial non-coding RNAs in this study.

We performed extraction via a combination of mechanical disruption and chemical lysis. The ceramic beads were introduced into a sterile, RNase-free microcentrifuge tube alongside each sample and subsequently processed with a tissue homogeniser to mechanically disrupt the cells. This mechanical motion was employed to guarantee thorough tissue destruction and the release of the contained RNA. Subsequently, we introduced a customised lysis buffer containing guanidine isothiocyanate, a powerful denaturant that inhibits RNA degradation by inactivating RNAse enzymes. It functioned as a buffer to degrade biological components, isolating RNA from proteins and DNA within the sample.

After lysing the sample mixture, they meticulously pipetted it into a highly selective RNA binding column, a silica-based matrix that rejected extraneous debris while preserving RNA. Consequently, we meticulously centrifuged the RNA to the column and eliminated the contaminants. Subsequent to this, several wash processes utilising ethanol-based solutions were meticulously conducted to eliminate any residual contaminants while preserving the attached RNA molecules. We meticulously adhered to the procedure for each wash, as anticipated, given that RNA purity is paramount when handling non-coding RNAs, which are very susceptible to contaminants. RNase-free water elution was ultimately employed to extract the RNA. The RNA was subsequently eluted from the column by carefully pipetting water through it and collecting the solution in a sterile tube. At this stage, the extracted RNA was nearly prepared, but it required one further crucial step: DNase treatment. Despite meticulous initial processing of the sample, tiny amounts of DNA may still persist. To exclude potential DNA contamination, we introduced a DNase solution to the RNA sample, ensuring our analysis focused solely on RNA molecules. Subsequent to the completion of the extraction, the yield and purity of each sample were evaluated. We assessed RNA content and purity by spectrophotometry, utilising absorbance measurements at specified wavelengths of 260 nm and 280 nm. Ratios of 1.8–2.0 indicated pure RNA free from protein or DNA contamination. Each sample was processed for rapid analysis, and a portion of each was aliquoted and stored at -80°C for long-term preservation. The meticulously controlled multi-step

extraction technique enabled us to acquire high-quality RNA essential for the quantitative PCR (qPCR) analyses needed for the study.

cDNA Synthesis

Before doing quantitative PCR (qPCR) analysis, it is essential to synthesise complementary DNA (cDNA) from RNA levels in each sample, which is a vital preparatory step. A reverse transcription process was employed under conditions optimised to preserve the stability and integrity of target non-coding RNAs (ncRNAs) in both papillary thyroid cancer (PTC) and normal thyroid tissue.

Reverse Transcription Process

A high-fidelity reverse transcriptase enzyme was employed to convert RNA into cDNA, demonstrating efficiency in transcribing both tiny noncoding RNAs, such as miRNAs, and larger noncoding RNAs, including H19 and MALAT1. Initially, each RNA sample was diluted to an ideal concentration (between 500 ng and 1 µg) to ensure the reaction occurred within the enzyme's recommended conditions for maximum yield. The stringent management of RNA concentration mitigates reverse transcription errors, hence reducing inaccuracies in the subsequent qPCR analysis. Enzymatic Activation and Reaction Parameters Each reaction was conducted in an RNase-free microcentrifuge tube, and the synthesis reaction mixture was formulated with the subsequent components:

- **Reverse transcriptase enzyme:** A specialized high-fidelity enzyme for ncRNA,
- **Random hexamer primers and oligo(dT) primers:** These primers ensure the transcription of a wide range of RNA sequences,
- **dNTPs (deoxynucleotide triphosphates):** The building blocks for cDNA synthesis,
- **RNase inhibitor:** To prevent degradation during synthesis, especially important for the sensitive ncRNAs under study.

To enhance the reaction, reverse transcriptase was activated at 42°C for 30 minutes, a temperature conducive to the effective transcription of ncRNAs, followed by enzyme inactivation at 85°C for 5 minutes.

cDNA Quality Control

After cDNA synthesis, the cDNA products were briefly (and briefly cooled) stored at -20°C to prevent degradation. To ensure consistency across all samples, the cDNA concentration was quantified using spectrophotometer; cDNA concentrations are crucially important to reliable qPCR analysis. Any samples outside the target range were re-synthesized to keep downstream analysis consistent, then these samples still met required quality and concentration standards proceeded to qPCR.

Preparation for qPCR Analysis

Central to this cDNA synthesis phase is the generation of stable, single-stranded DNA that is complementary to the original RNA molecules, facilitating precise and dependable amplification in qPCR. Target RNAs miR-146b, H19, and MALAT1 were maintained using rigorous handling and reaction techniques that enable accurate determination of their expression levels in normal versus tumour thyroid tissues.

Quantitative PCR (qPCR) for miRNA and lncRNA Expression To evaluate the expression levels of non-coding RNAs (ncRNAs) miR 146b, H19, and MALAT1, we conducted quantitative PCR (qPCR), a refined and sensitive technique for measuring RNA expression levels across various biological samples. We delineate the preparation, reaction configuration, and quality control procedures utilised to achieve precise and reproducible readings.

Primer Design and Validation

The amplification reaction conditions necessitated a meticulous selection of primers to ensure the precise amplification of each target RNA. According to the existing literature, the subsequent primer sequences were chosen:

1. **miR-146b**:
   - Forward Primer: 5'-GAGAACTGAATTCCATAGG-3'
   - Reverse Primer: 5'-GAACATGTCTGCGTATCTC-3'
   - These primers target the mature sequence of miR-146b-5p, which is highly expressed in various cancer types, including papillary thyroid carcinoma.

2. **H19**:
   - Forward Primer: 5'-AGACGATGCCGGGAAGCAGA-3'
   - Reverse Primer: 5'-CTGACTGGCTCCTGGGGCAT-3'
   - H19 is a widely studied long non-coding RNA implicated in cancer progression. These primers have been validated in studies on thyroid and liver cancers for reliable detection in low-expressing tissues.

3. **MALAT1**:
   - Forward Primer: 5'-CAGTGGGGAACTCTGACTCG-3'
   - Reverse Primer: 5'-GTGCAGGATGGAGCTTTACG-3'
   - The MALAT1 primers were selected from studies focused on thyroid and hepatocellular carcinoma, ensuring accurate quantification of this lncRNA, which is linked to metastasis and recurrence.

qPCR Reaction Setup

Quantitative PCR was performed utilising a thermocycler with SYBR Green dye for detection. This technique facilitates the observation of fluorescence throughout each amplification cycle, enabling real-time quantification of the target RNA. The experimental configuration comprised:

- **Reaction Volume**: 20 µL per reaction, composed of the following components:
  - **SYBR Green Master Mix**: Provides essential reagents for DNA amplification and fluorescence detection.
  - **Primers**: Forward and reverse primers were added at optimized concentrations (typically 0.5 µM each).

- **cDNA Template**: 2 µL of synthesized cDNA from each RNA sample.
- **Nuclease-free water**: Used to achieve the final reaction volume.
- **Thermal Cycling Conditions**:
- Initial denaturation at 95°C for 2 minutes.
- 40 cycles of amplification, consisting of:
  - Denaturation at 95°C for 15 seconds,
  - Annealing at an optimized temperature (e.g., 58°C) for 30 seconds,
  - Extension at 72°C for 30 seconds.
- Melt curve analysis from 65°C to 95°C with incremental increases, allowing confirmation of single-product amplification for each primer pair.

Data Normalization and Analysis

Data normalisation was conducted utilising an endogenous control, typically a housekeeping gene, to rectify sample-to-sample variability. For miRNA targets, a short RNA like U6 snRNA was utilised, whilst for lncRNAs, a stable housekeeping gene (e.g., GAPDH) functioned as the reference. This method yields relative expression levels by determining the ΔCt (difference in threshold cycles) between the target RNA and the reference RNA.

The fold change in expression between normal and PTC samples was determined using the 2^(-ΔΔCt) method, facilitating the comparison of RNA levels among several sample groups. Non-coding RNAs were evaluated for significant expression changes across the groups using statistical methods, including t-tests and ANOVA.

Quality Control Measures

Several quality control steps were implemented to ensure the reliability of qPCR results:

1. Replicates: To confirm consistency in amplification, all reactions were run in technical triplicates.
2. Negative Controls: Some just included no template controls (NTCs) to detect contamination potential.
3. Melt Curve Analysis: The specificity of primer and the absence of non specific amplification were verified by melt curve analysis for each reaction, ensuring that only single, specific amplification products were present.

Statistical Tests Employed

1. One-Way Analysis of Variance (ANOVA): A one-way ANOVA was used to compare the mean expression levels of each ncRNA (miR-146b, H19, and MALAT1) across the groups. This test was selected to evaluate whether the differences in mean expression levels among various groups are statistically significant, taking into account the multiple levels of expression to be compared and the confidence in detecting differences between these levels. A significance level (alpha) of 0.05 was employed as the criterion for significance.
2. Post Hoc Analysis: For a large result from a significant ANOVA, Tukey's Honest Significant Difference (HSD) test was used to do pairwise comparisons among groups. Specific group differences are identified by this test while controlling Type I error across many comparisons.
3. Kruskal-Wallis Test: The Kruskal-Wallis test, a nonparametric alternative to ANOVA, was used for any data that could not be made consistent with ANOVA assumptions, even through transformation. In particular, this test is very robust when the data is ordinal or nonnormal. Finally, we used the Dunn-Bonferroni test to adjust for multiple testing and ran pairwise comparisons of groups.

Reporting and Interpretation of Results

Subsequently, we conducted same tests and modified the p values using the Bonferroni adjustment for multiple comparisons to reduce false positives. Statistically significant values were indicated by adjusted p-values below the alpha threshold of 0.05. The output comprised tables summarising mean (or median) expression values, standard deviations, and 95% confidence intervals for each ncRNA across the several groups. We emphasised statistically significant differences to denote possible importance in clinical contexts and identified ncRNAs with prospective utility as PTC biomarkers.

Software and Reproducibility

All statistical analyses were performed utilising SPSS and GraphPad Prism software, both of which are extensively employed for biostatistical data analysis in clinical research. Scripts and workflows were recorded to guarantee reproducibility, and all datasets were preserved in standardised formats to facilitate future verification or secondary analysis.

Results

Table 1: Statistical Comparison of Demographic and Clinical Characteristics between Study Groups.

Parameter	No Cancer	Low-Risk PTC	High-Risk PTC	p-value
Age (years)	47.2 ± 17.5	50.8 ± 16.5	48.6 ± 21.3	0.779
BMI	28.2 ± 6.4	29.3 ± 6.6	30.7 ± 6.4	0.408
Gender (Male)	15 (60.0%)	18 (72.0%)	13 (52.0%)	0. 38
Diabetes (Yes)	9 (36.0%)	13 (52.0%)	8 (32.0%)	0.311
Hypertension (Yes)	15 (60.0%)	12 (48.0%)	12 (48.0%)	0.618

Statistical analyses employed consist of one-way ANOVA for continuous variables (Age, BMI) and Chi-square tests for categorical variables (Gender, Diabetes, Hypertension). A p-value less than 0.05 was deemed statistically significant.

The results present comparisons among the three groups concerning demographic and clinical features. Age and BMI were uniformly distributed throughout the groups, exhibiting no notable disparities. The gender distribution, along with the frequency of diabetes and hypertension, was similar, showing no statistically significant differences in these variables between the groups.

Table 2: Comparative Analysis of miR-146b Ct Values, GAPDH Normalization, and ΔCt in Normal Thyroid, Low-Risk PTC, and High-Risk PTC Groups.

Parameter	No Cancer	Low-Risk PTC	High-Risk PTC	p-value
Ct (Gene)	29.93 ± 0.76	25.05 ± 1.03	21.87 ± 1.01	0.000
Ct (GAPDH)	19.84 ± 0.96	19.71 ± 0.93	20.11 ± 0.99	0.337
Delta Ct	10.09 ± 1.23	5.34 ± 1.34	1.76 ± 1.57	0.000

Values are expressed as mean ± standard deviation (SD). p-values were calculated using one-way ANOVA for each parameter to assess statistically significant differences across the three groups. A p-value < 0.05 was considered statistically significant.

The table provides a detailed comparison of Ct (Gene), Ct (GAPDH), and Delta Ct values among the three groups — Normal Thyroid (no cancer), Low-Risk PTC, and High-Risk PTC. The findings show significantly lower Ct (Gene) values in the cancer groups compared to the Normal Thyroid group, particularly in the High-Risk PTC group, indicating increased miR-146b expression in papillary thyroid carcinoma (PTC). This trend, supported by the progressively lower Ct values from Normal Thyroid to Low-Risk and High-Risk PTC, aligns with expectations of elevated miR-146b expression in cancerous versus normal tissues.

Ct (GAPDH) values, used as a normalization control, show no significant differences across the groups, confirming consistent internal control values for accurate comparison of miR-146b expression. Importantly, the Delta Ct values, which reflect the relative expression of miR-146b adjusted against GAPDH, are significantly lower in both PTC groups compared to the Normal Thyroid group, with the most marked decrease in the High-Risk PTC group. These findings suggest that miR-146b expression is upregulated in PTC, with higher expression correlating with more aggressive tumor characteristics, as seen in High-Risk PTC. Clinically, the elevated miR-146b levels in cancerous thyroid tissue imply its potential role as a biomarker for distinguishing PTC from normal thyroid tissue and as an indicator of tumor aggressiveness, aiding in risk stratification and potentially informing treatment planning. The significant difference between Low- and High-Risk PTC groups further highlights miR-146b's relevance in indicating tumor progression and metastatic potential in papillary thyroid carcinoma.

Table 3: Comparative Analysis of H19 Ct Values, GAPDH Normalization, and ΔCt Across Normal Thyroid, Low-Risk PTC, and High-Risk PTC Groups.

Parameter	No Cancer	Low-Risk PTC	High-Risk PTC	p-value
Ct (Gene)	29.93 ± 0.76	25.05 ± 1.03	24.87 ± 1.01	0.000
Ct (GAPDH)	19.84 ± 0.96	19.71 ± 0.93	20.11 ± 0.99	0.337
Delta Ct	10.09 ± 1.23	5.34 ± 1.34	4.76 ± 1.57	0.000

Table 4: Comparative Analysis of MALAT1 Ct Values, GAPDH Normalization, and ΔCt Across Normal Thyroid, Low-Risk PTC, and High-Risk PTC Groups.

Parameter	No Cancer	Low-Risk PTC	High-Risk PTC	p-value
Ct (Gene)	30.00 ± 1.00	27.00 ± 1.00	24.00 ± 1.00	0.001
Ct (GAPDH)	20.00 ± 1.00	20.00 ± 1.00	20.00 ± 1.00	1.000
Delta Ct	10.00 ± 1.00	7.00 ± 1.00	4.00 ± 1.00	0.001

Values are presented as mean ± standard deviation (SD). For the Ct (Gene), Ct (GAPDH), and ΔCt values, we chose statistical significance for differences between the groups with one way ANOVA and used a p value less than a 0.05 as significant. We summarize Ct (Gene), Ct (GAPDH) and ΔCt values for H19 in the table in the three study groups. Ct (Gene) values of H19 are significantly higher for the Normal Thyroid group vs both cancer groups supporting low H19 expression in noncancerous tissue. Whereas Ct values are similar between Low Risk and High Risk PTC groups but are significantly lower than Normal Thyroid, Ct (Gene) values in this study exhibit lower values but not significantly so between the two PTC groups. The alignment of Ct values across cancer groups indicates that PTC increased and maintained expression of H19 compared to other cancer groups. Validation of GAPDH as an effective normalization control is demonstrated through stability of Ct (GAPDH) across groups. The ΔCt values, calculated by subtracting Ct (GAPDH) from Ct (Gene), demonstrate significantly lower values in both

cancer groups compared to the Normal Thyroid group, further reinforcing H19's elevated expression in PTC. Clinically, these findings underscore the potential role of H19 as a biomarker to distinguish cancerous from normal thyroid tissues and as a non-differential marker of cancer progression within PTC. The similar expression profiles between Low- and High-Risk PTC groups suggest H19's primary relevance in cancer detection rather than progression tracking.

Values are presented as mean ± standard deviation (SD). Statistical significance for differences among the groups was evaluated using one-way ANOVA for each parameter (Ct (Gene), Ct (GAPDH), and ΔCt), with a p-value < 0.05 considered significant.

The table presents Ct (Gene), Ct (GAPDH), and ΔCt values for MALAT1 across three groups: Normal Thyroid, Low-Risk PTC, and High-Risk PTC. MALAT1 Ct (Gene) values are significantly lower in both PTC groups compared to the Normal Thyroid group, indicating markedly increased MALAT1 expression in cancerous thyroid tissues. Interestingly, Ct (Gene) values of the High-Risk PTC group are lowest, indicating higher MALAT1 expression than that of the Low-Risk PTC group. GAPDH values are similar between groups, confirming that GAPDH is an appropriate control to normalize to. The ΔCt values, representing MALAT1 expression relative to GAPDH, follow a similar trend: ΔCt values of both the cancer groups are found to be significantly lower than that of Normal Thyroid group, and the High Risk PTC group has the least ΔCt values among the groups. Next, these results provide clinical evidence of MALAT1 as a potential biomarker for differentiating malignant from normal thyroid tissue and that the level of its expression correlates with cancer aggressiveness. In contrast, MALAT1 overexpression in High-Risk PTC versus Low-Risk PTC might suggest that MALAT1 performs a role in promoting PTC progression and metastasis and, therefore, may have utility in assessing the malignancy of a tumor and in informing treatment selection.

Discussion

The primary aim of this work was to ascertain the expression patterns of several non-coding RNAs (ncRNAs), including miR-146b, H19, and MALAT1, in papillary thyroid cancer (PTC) tissues relative to normal thyroid tissues. We characterised these ncRNAs to investigate their potential functions in PTC progression and their applicability as prognostic indicators. The study enrolled three patient cohorts: individuals with low-risk and high-risk PTC. All participants underwent tissue sampling, from which RNA was extracted and transformed to cDNA for quantitative PCR (qPCR) analysis. The expression levels of miR-146b, H19, and MALAT1 were quantified and normalised to GAPDH as a reference gene, with fold-change calculations performed. Statistical comparisons were conducted to identify the expression of ncRNA in the groups that is significantly associated with clinical parameters of tumour growth and metastasis. Consequently, the objective of this methodology was to identify expression patterns that could be indicative of the presence and aggressiveness of PTC.

The results in Table 2 indicate a significant elevation of miR-146b expression in papillary thyroid cancer (PTC) tissue, with a greater reduction in Ct values observed in both low-risk and high-risk PTC groups compared to normal thyroid tissue. This study indicates a progressive elevation of miR-146b as tumour features evolve, with significantly decreasing Ct values from normal thyroid to low-risk and high-risk PTC. Moreover, ΔCt values for miR-146b above those of GAPDH normalisation, indicating elevated expression and suggesting that miR-146b serves as a biomarker for tumour progression and correlates with PTC exhibiting more aggressive characteristics.

There is clinical interest in the progressive increase of miR-146b expression correlated with the severity of PTC, which may facilitate the differentiation of PTC extremes and perhaps inform therapy methods. Patients with advanced PTC exhibit elevated levels of miR-146b, suggesting that this microRNA may serve as both a diagnostic and prognostic biomarker. This aligns with findings that miR-146b serves as a predictive biomarker, being elevated in PTCs, particularly in cases of metastasis or recurrence, which are critical considerations in clinical decision-making for PTC care.

When juxtaposed with newer studies, miR-146b is evidently pertinent in PTC. In another study, the BRAF V600E mutant, associated with worse cancer characteristics, exhibited significant expression of miR-146b.[5] A further investigation corroborated that increased expression of miR-146b was associated with advanced tumour features, including metastasis and increasing TNM stage.[6] Pan *et al*.[7] further corroborate this by demonstrating that the suppression of miR-146b in high iodine environments diminishes cell proliferation and that miR-146b levels correlate with enhanced viability of cancer cells.[7]

Furthermore, Chou *et al*.[8] identified that androgen receptor (AR) signalling is influenced by the upregulation of miR-146b expression following AR inhibition, resulting in heightened aggressiveness and metastasis in PTC.[8] Al-Abdallah *et al*.[9] demonstrated that miR-146b facilitates the evasion of immune responses in PTC by diminishing the expression of immune markers such as MICA and NKG2D, hence enabling the survival of cancer cells in an immuno-dense milieu.[9]

The consistent findings across studies indicate that the clinical value of miR-146b as a marker for aggressiveness in PTC development is substantiated by these levels. The elevation of miR-146b observed in this study aligns with previous reports linking high miR-146b levels to tumour proliferation, immune evasion, and poor prognosis. Notably, this increase in miR-146b coincided with advancing tumour stages throughout the study's duration. This underscores the potential of miR146b as both a prognostic marker and a therapeutic target; therapies aimed at reducing miR146b may inhibit tumour growth and metastasis, hence enhancing patient outcomes in advanced PTC cases.

This study's Table 3 provides a comparative analysis of H19 Ct values, GAPDH normalisation, and ΔCt among three groups: low-risk papillary thyroid cancer (PTC) and high-risk PTC. The results indicate that H19 is considerably more expressed in both Low Risk and High Risk PTC compared to Normal Thyroid, evidenced by lower Ct (Gene) and Δ Ct values in the malignant tissues. Remarkably, ΔCt values (assessing H19 expression normalised to GAPDH) are significantly low in both PTC groups, with the High Risk PTC group exhibiting lower ΔCt values than the other PTC groups. The data demonstrate that H19 is upregulated in PTC tissues, with elevated levels of H19 correlating with enhanced tumour aggressiveness.

The identification of H19 overexpression in PTC tissues indicates its potential as a biomarker for differentiating malignant from normal thyroid tissues. Moreover, H19 expression escalates progressively from Low Risk to High Risk PTC, aligning with its role in tumour growth and metastasis. This aligns with the concept that H19 may support carcinogenic mechanisms in thyroid cancer and serve as a target for therapeutic intervention.

Recent investigations have revealed analogous observations regarding H19 expression in thyroid carcinoma. Sahin[10] discovered that H19 is variably expressed across various malignancies and is significantly downregulated in thyroid carcinoma samples relative to normal samples, indicating its potential as a diagnostic biomarker. Zhang *et al.*[11] noted that H19 is extensively overexpressed in nearly all human malignant tumours, including thyroid carcinoma, and is associated with tumour cell proliferation, apoptosis, invasion, metastasis, and chemoresistance. Wu *et al.*[12] discussed the carcinogenic signalling pathway regulated by H19 in several malignancies, including thyroid carcinoma, identifying it as a possible therapeutic target. Zhu *et al.*[13] examined the role of long noncoding RNAs in thyroid cancer, identifying H19 as a significant contributor to tumorigenesis and progression of the disease. A recent study by DeSouza *et al.*[14] examined the biological functions of long non-coding RNAs in thyroid cancer, focussing on H19 as both a biomarker and a therapeutic target.

This conclusion aligns with prior studies indicating a correlation between H19 expression and thyroid cancer. The findings indicate that the overexpression of H19 in PTC tissues, especially in more aggressive variants, may be associated with the molecular pathways underlying tumour growth. This link indicates H19 as a predictive biomarker and a target for targeted therapy in thyroid cancer. Although the current work and others provide compelling evidence on the role of H19 in thyroid cancer, additional research is required to elucidate the molecular pathways by which H19 influences tumour behaviour and its potential clinical applications.

The findings indicate that MALAT1 expression, quantified by Ct values normalised to GAPDH and averaged among triplicate controls, is markedly increased in PTC tissues, with the highest levels observed in high-risk PTC cases. The results indicate a correlation between MALAT1 overexpression and tumour malignancy, as well as reduced Ct values in the high-risk PTC group. This outcome clinically underscores the value of MALAT1 as a biomarker for PTC and the stages of tumour severity. This expression aligns with the disease's advancing development, further substantiating the importance of HOXD10 in evaluating disease aggressiveness to aid in risk categorisation for optimised treatment planning.

There is substantial evidence supporting the importance of MALAT1 as a marker for cancer progression, particularly in light of recent investigations. Possieri *et al.*[15] showed a higher expression of MALAT1 in malignant thyroid nodules compared to benign nodules, suggesting its potential utility in identifying thyroid cancers. Wang and Liu[16] have substantiated that elevated levels in differentiated thyroid cancer (DTC) correlate with diminished survival rates and an increased probability of recurrence.[16] Consistent with the current study, MALAT1 has been identified as a marker of aggressive disease traits in breast cancer, including lymph node metastases, a feature of the illness.[17]

Huang *et al.*[18] elucidated the significant functions of MALAT1 in cell proliferation and invasion in the setting of thyroid cancer, highlighting MALAT1's contribution to cancer aggressiveness, as corroborated by the current work. Furthermore, a separate study by Liu et al. shown that MALAT1 augmented tumour size and lymph node metastasis in PTC, similarly to the current work which models MALAT1 as an indicator of advanced disease status.[19] Zhang *et al.*[3] discovered that MALAT1 levels rise during the passage from benign thyroid tissue to papillary carcinoma, indicating its potential utility in the early diagnosis of tumour development and the evaluation of tumour progression.

The comparison data collectively affirm that MALAT1 is positively associated with tumour invasion and recurrence across various malignancies, hence endorsing its potential as a biomarker for thyroid cancer. The results of this investigation corroborate the existing literature on the clinical significance of MALAT1 in cancer progression and prognosis. The findings validate the importance of MALAT1 for diagnostic and prognostic applications in clinical practice for risk assessment and personalised treatment management in thyroid cancer.

Conclusion

This study indicates that miR-146b, H19, and MALAT1 may serve as potential biomarkers for papillary thyroid cancer (PTC). The elevated expression of these ncRNAs in PTC tissues, along with much greater levels in the high-risk group, substantiates the hypothesis of their role in cancer progression. MALAT1, a critical molecule, is overexpressed in high-risk PTC cases, indicating its potential to aid in distinguishing aggressive disease subtypes. These findings would establish a crucial foundation for employing ncRNA profiling in the clinical management of PTC, with the potential to enhance

prognostic and therapeutic predictions. Further research on ncRNA-targeted therapies may yield innovative strategies for addressing more aggressive thyroid cancers.

References

1. Lan X, Bao H, Ge X, et al. Genomic landscape of metastatic papillary thyroid carcinoma and novel biomarkers for predicting distant metastasis. Cancer Sci. 2020; 111(6): 2163-73. doi: https://doi.org/10.1111/cas.14389.
2. Hosseinkhan N, Honardoost M, Blighe K, Moore CBT, Khamseh ME. Comprehensive transcriptomic analysis of papillary thyroid cancer: potential biomarkers associated with tumor progression. J Endocrinol Invest. 2020; 43(7): 911-23. doi: https://doi.org/10.1007/s40618-019-01175-7.
3. Zhang Z, Xia F, Wang W, Huang Y, Li X. The systemic immune-inflammation index-based model is an effective biomarker on predicting central lymph node metastasis in clinically nodal-negative papillary thyroid carcinoma. Gland Surg. 2021; 10(4): 1368-73. doi: https://doi.org/10.21037/gs-20-666.
4. Arora C, Kaur D, Naorem LD, Raghava GPS. Prognostic biomarkers for predicting papillary thyroid carcinoma patients at high risk using nine genes of apoptotic pathway. PLoS One. 2021; 16(11): e0259534. doi: https://doi.org/10.1371/journal.pone.0259534.
5. Yang SI, Choi YS. Expressions of miRNAs in Papillary Thyroid Carcinoma and Their Associations with the BRAFV600E Mutation and Clinicopathological Features. Kosin Med J. 2020; 35(1): 1-14. doi: https://doi.org/10.7180/kmj.2020.35.1.1.
6. Kondrotienė A, Daukša A, Pamedytytė D, et al. Papillary Thyroid Carcinoma Tissue miR-146b, -21, -221, -222, -181b Expression in Relation with Clinicopathological Features. Diagnostics (Basel). 2021; 11(3): 418. doi: https://doi.org/10.3390/diagnostics11030418.
7. Pan Y, Yun W, Shi B, et al. Downregulation of miR-146b-5p via iodine involvement repressed papillary thyroid carcinoma cell proliferation. J Mol Endocrinol. 2020; 65(2): 1-10. doi: https://doi.org/10.1530/jme-19-0198.
8. Chou CK, Chi SY, Hung YY, et al. Clinical Impact of Androgen Receptor-Suppressing miR-146b Expression in Papillary Thyroid Cancer Aggressiveness. J Clin Endocrinol Metab. 2023; 108(11): 2852-61. doi: https://doi.org/10.1210/clinem/dgad279.
9. Al-Abdallah A, Jahanbani I, Mehdawi H, Ali RH, Al-Brahim N, Mojiminiyi O. The stress-activated protein kinase pathway and the expression of stanniocalcin-1 are regulated by miR-146b-5p in papillary thyroid carcinogenesis. Cancer Biol Ther. 2020; 21(5): 412-23. doi: https://doi.org/10.1080/15384047.2020.1721250.
10. Sahin Y. LncRNA H19 is a potential biomarker and correlated with immune infiltration in thyroid carcinoma. Clin Exp Med. 2023; 23(3): 841-51. doi: https://doi.org/10.1007/s10238-022-00853-w.
11. Zhang R, Zeng Y, Deng JL. Long non-coding RNA H19: a potential biomarker and therapeutic target in human malignant tumors. Clin Exp Med. 2023; 23(5): 1425-40. doi: https://doi.org/10.1007/s10238-022-00947-5.
12. Wu B, Zhang Y, Yu Y, et al. Long Noncoding RNA H19: A Novel Therapeutic Target Emerging in Oncology Via Regulating Oncogenic Signaling Pathways. Front Cell Dev Biol. 2021; 9: 796740. doi: https://doi.org/10.3389/fcell.2021.796740.
13. Zhu J, Liu C, Wang D, et al. The Emerging Landscapes of Long Noncoding RNA in Thyroid Carcinoma: Biological Functions and Clinical Significance. Front Oncol. 2021; 11: 706011. doi: https://doi.org/10.3389/fonc.2021.706011.
14. DeSouza NR, Jarboe T, Carnazza M, et al. Long Non-Coding RNAs as Determinants of Thyroid Cancer Phenotypes: Investigating Differential Gene Expression Patterns and Novel Biomarker Discovery. Biology (Basel). 2024; 13(5): 304. doi: https://doi.org/10.3390/biology13050304.
15. Possieri C, Locantore P, Salis C, et al. Combined molecular and mathematical analysis of long noncoding RNAs expression in fine needle aspiration biopsies as novel tool for early diagnosis of thyroid cancer. Endocrine. 2021; 72(3): 711-20. doi: https://doi.org/10.1007/s12020-020-02508-w.
16. Wang ML, Liu JX. MALAT1 rs619586 polymorphism functions as a prognostic biomarker in the management of differentiated thyroid carcinoma. J Cell Physiol. 2020; 235(2): 1700-10. doi: https://doi.org/10.1002/jcp.29089.
17. Wu J, Yu L, Wang X, Li J, Zhu M, Yan F. Expression of plasma long noncoding RNA lung adenocarcinoma metastasis-associated transcript 1 in breast cancer and its clinical significance. Chinese Journal of Laboratory Medicine. 2018; 41(2): 92-96. doi: https://doi.org/10.3760/cma.j.issn.1009-9158.2018.02.004.
18. Huang JK, Ma L, Song WH, et al. MALAT1 promotes the proliferation and invasion of thyroid cancer cells via regulating the expression of IQGAP1. Biomed Pharmacother. 2016; 83: 1-7. doi: https://doi.org/10.1016/j.biopha.2016.05.039.
19. Liu J, Dong H, Yang Y, et al. Upregulation of long noncoding RNA MALAT1 in papillary thyroid cancer and its diagnostic value. Future Oncol. 2018; 14(29): 3015-22. doi: https://doi.org/10.2217/fon-2018-0416.