

A Comparative Transcriptomic Analysis of Pathogenic Mechanisms between Papillary Thyroid Carcinoma and Hashimoto's Thyroiditis

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Abstract: Papillary thyroid carcinoma (PTC) and Hashimoto's thyroiditis (HT) appear antagonistic but often occur simultaneously. This study integrates four GEO datasets to compare gene expression differences among PTC, HT, PTC-HT, and control thyroid tissues (Control), revealing similarities and differences in gene expression. Results show significant differences in 202 genes among the three conditions, with consistent upregulation/downregulation trends, primarily involving inflammation and chemokines (e.g., CXCL8, CXCL9, CXCL10, IL8). Cells in PTC and HT are in a state of chronic inflammatory activation and are unresponsive to p53 and FAS-induced apoptotic signals. Analysis of differentially expressed genes between PTC and HT reveals that several extracellular matrix pathway genes (e.g., FN1, LAMB1, LAMC1) have opposite expression trends in the two diseases, and the FN1-DUSP6-ETV5-NFATC1/3 signaling axis may drive phenotypic heterogeneity by regulating T cell immune responses. These findings provide new insights into the molecular mechanisms and interrelationship of PTC and HT.

Keywords: Papillary thyroid carcinoma; Hashimoto's thyroiditis; Expression profile comparative analysis;

Introduction

Papillary thyroid carcinoma (PTC) is considered the most common type of thyroid cancer, characterized by unique histological features and generally favorable prognosis. However, the molecular basis of its pathogenesis, especially in relation to autoimmune diseases such as Hashimoto's thyroiditis (HT), remains unclear. Hashimoto's thyroiditis is an autoimmune disease marked by lymphocytic infiltration of the thyroid gland¹. Its potential role as a precursor or risk factor for PTC has been a focus of research. Multiple studies have shown that Hashimoto's thyroiditis frequently coexists with PTC, often manifesting as multifocal carcinogenesis, but with lower clinical staging compared to thyroid cancer without thyroiditis^{2,3}.

With the development of bioinformatics, powerful tools have been provided to explore the complex gene expression profiles associated with these diseases. Using comprehensive bioinformatics analysis, differentially expressed genes (DEGs) and key molecular pathways can be identified, which may promote the development and progression of PTC in the context of HT. Such analyses reveal significant associations between specific gene expression patterns and the pathogenesis of PTC, providing insights into potential biomarkers and therapeutic targets^{4,5}.

In this study, we aim to conduct a comprehensive bioinformatics analysis comparing the expression profiles of PTC and HT. By utilizing high-throughput sequencing data and advanced computational methods, key differentially expressed genes and pathways under these conditions will be identified. This approach will deepen our understanding of the molecular interactions between PTC and HT, facilitating the discovery of novel biomarkers for early diagnosis and targeted therapy⁶.

Materials and Methods

Microarray Data, Patients, Tissue Collection

From August 2024 to February 2025, a total of 10 tissue samples were collected, including 3 Hashimoto's, 6 papillary carcinomas, and 1 para-carcinoma tissue, from Changxing County People's Hospital. The patients' ages ranged from 27 to 68 years, including 1 male and 8 female patients.

This study was approved by the Ethics Committee of Changxing County People's Hospital. Written informed consent was obtained from all participants or their legal guardians.

Data Acquisition

Relevant studies on Hashimoto's Thyroiditis (HT) and Papillary Thyroid Carcinoma (PTC) were collected from the GEO database. Based on data consistency considerations, only microarray data was selected, using keywords Hashimoto's Thyroiditis, Papillary Thyroid Carcinoma, and PTC, referencing literature with PMIDs 25306886 and 34313737. The selected datasets were: ① GSE138198 (36 samples total), selecting 13 HT samples, 12 PTC samples, 8 PTC_HT samples, and 3 normal thyroid samples (TN) for control. A total of 36 samples were selected from GSE138198 (see batch1 in Appendix Table 1). ② GSE29315 (71 samples total), selecting 6 HT samples and 22 PTC samples. A total of 28 samples were selected from GSE29315 (see batch2 in Appendix Table 1). ③ GSE33630 (105 samples total), selecting 49 PTC samples and 45 normal thyroid tissue samples for control. A total of 94 samples were selected from GSE33630 (see batch3 in Appendix Table 1). ④ GSE66463 (18 samples total), selecting 8 PTC samples. A total of 8 samples were selected from GSE66463 (see batch4 in Appendix Table 1). These datasets were all obtained using the Affym-

etrix Human Gene/Genome Array platform. Finally, a total of 166 samples were pooled for the study, including 19 HT cases, 91 PTC cases, 8 PTC_HT cases, and 48 control cases.

Differential Gene Expression Analysis

To eliminate the impact of batch effects on gene expression data, we used the sva (Surrogate Variable Analysis) package in R for data correction⁷. J. T. </author><author>Johnson, W. E.</author><author>Parker, H. S.</author><author>Jaffe, A. E.</author><author>Storey, J. D.</author></authors></contributors><auth-address>Department of Biostatistics, JHU Bloomberg School of Public Health, Baltimore, MD, USA. jleek@jhsph.edu</auth-address><titles><title>The sva package for removing batch effects and other unwanted variation in high-throughput experiments</title><secondary-title>Bioinformatics</secondary-title></titles></periodical><full-title>Bioinformatics</full-title></periodical><pages>882-3</pages><volume>28</volume><number>6</number><edition>2012/01/20</edition><keywords><keyword>Gene Expression Profiling</keyword><keyword>Genomics</keyword><keyword>High-Throughput Nucleotide Sequencing</keyword><keyword>Humans</keyword><keyword>Regression Analysis</keyword><keyword>*Software</keyword><keyword>Urinary Bladder Neoplasms/genetics</keyword></keywords><dates><year>2012</year><pub-dates><date>Mar 15</date></pub-dates></dates><isbn>1367-4811 (Electronic. The combat function in the sva package was applied, using Bayesian methods to adjust the data and eliminate systematic biases from different batches. After correction, differentially expressed genes (DEGs) were identified between the control group and PTC, HT, PTC_HT groups using the ‘limma’ package. The analysis workflow is shown in Figure 1, with comparison conditions and parameters also standardized in the figure. Volcano plots were generated using the ‘ggplot2’ package to visualize DEGs, and heatmaps of the top 50 DEGs were created using the ‘Pheatmap’ package. Differential gene clustering analysis was performed using the clusterProfiler package in R and manually beautified.

Similarly, all 166 samples were analyzed using the WGCNA package in R, with a minimum module size of 30 and a minimum module dissimilarity threshold of 0.15. For different disease-related color groups, HubGenes were screened using parameters of |GS|>0.2 and |MM|>0.5. The same methods were used for gene differential volcano plots, heatmaps, and gene clustering analysis using the clusterProfiler package. Protein-protein interaction (PPI) analysis was performed using the STRING database (<https://string-db.org/>).

Organizational Real-time Fluorescence Quantitative (RT-qPCR) and WB Analysis for Verification

Total RNA was extracted using the total RNA extraction reagent (Solarbio, Cat. No. R1200) according to the manufacturer’s instructions. cDNA reverse transcription was performed using microRNA RT Master Mix for qPCR III (poly A) (MCE, Cat. No. HY-K0512) as per the manual. The qPCR reaction was pre-

pared using PerfectStart Green qPCR SuperMix Kit (with dye II, TransGen, Cat. No. AQ602-01) according to instructions and run on the ABI Prism 7500 system (ABI). The expression level of target mRNA was normalized to β -actin as an internal reference, and the relative expression difference was calculated using the $2^{-\Delta\Delta Ct}$ method, calculating the fold change relative to the control group.

WB verification of collected samples was performed using antibodies from Bioproduct: NFATc3 (YP-mAb-01898); NFATc1 (YP-mAb-02243); ETV5 (H00002119-A01); UDSP6 (Self-made antibodies); FN1 (YP-mAb-17024); TP53 (YP-mAb-00474).

Results

Analysis of Common Differential Genes in Three Disease Tissues:

After batch correction, normalized gene expression data for all 166 samples were obtained (see Appendix 2). Using these data, comparisons were made between PTC_HT, PTC, and HT with Control tissues, followed by Pathway1 analysis as shown in the schematic diagram. Differential genes DEG1, DEG2, and DEG3 were obtained (see Appendix 3). Comparing PTC_HT with Control (DEG1, Figure 2B), 510 upregulated genes and 328 downregulated genes were identified. Comparing PTC with Control (DEG2, Figure 2A), 258 upregulated genes and 165 downregulated genes were found. Comparing HT with Control (DEG3, Figure 2C), 352 upregulated genes and 114 downregulated genes were identified. The intersection of DEG1, DEG2, and DEG3 in a Venn diagram revealed 202 genes differentially expressed in all three diseases. The expression trends of these differential genes, both upregulation and downregulation, were consistent across the three diseases (see Appendix 2). A heatmap of the top 50 genes with the largest expression differences (Figure 2E) showed clear separation between disease groups and the control group, but clustering among the three diseases was not distinct. This suggests that the gene expression profiles of these three diseases may be relatively similar.

KEGG clustering analysis was performed on the 202 common differential genes, and pathways with $q\text{Value} < 0.05$ were selected to draw the KEGG clustering diagram. It was found that genes related to various infectious diseases and complement pathways were altered, followed by changes in pathways related to p53 gene, lysosomes, and phagosomes associated with cell apoptosis. Finally, we also found significant changes in the expression of genes involved in cell adhesion and cytoskeleton pathways.

Displaying these pathways and their connecting key genes using a pathway-gene diagram (Figure 2G) revealed: genes related to infection and immune regulation were mostly upregulated, especially HLA genes associated with autoimmunity. Pro-apoptotic genes FAS and BID were upregulated (Figure 2G, H), mainly connecting p53 pathway, cisplatin resistance pathway, and multiple immune disease pathways, but their upregulation did not appear to cause apoptosis of immune system or cancer-related genes. We also separately investigated

important apoptosis-related genes TP53 (encoding p53, tumor suppressor gene), BCL2, and BCL2L2 (anti-apoptotic genes) in the four types of tissues (Figure 2H). TP53 gene was upregulated in all three disease tissues, and its induced anti-apoptotic genes BCL2 and BCL2L2 were downregulated. This activation of the apoptotic signaling pathway did not effectively inhibit the occurrence of cancer or autoimmunity.

Pairwise Comparison Analysis of Differential Genes in Three Diseases:

After analyzing the common differential genes in the three diseases, we also analyzed the common genes in PTC and HT, following Pathway2 and Pathway3 in the analysis flowchart. There were 1175 genes upregulated in both PTC and HT (Figure 3A). Clustering analysis of these genes revealed a focus on immune regulation and apoptosis systems. In addition to the pathways observed in Figure 2, pathways related to cell matrix such as “focal adhesion” and “adhesion molecules” were also upregulated (Figure 3B). After drawing the pathway-gene diagram (Figure 3C), we also found upregulation of numerous chemokines such as CXCL8, CXCL9, CXCL10, and pro-inflammatory factors like CD4 and IL8.

There were 1764 genes downregulated in both PTC and HT (see Figure 3D). KEGG clustering of these genes showed that the main downregulated pathways were metabolism-related, such as carbon, glucose, and amino acids (see Figure 3E). At the same time, some anti-apoptotic genes, such as the previously mentioned BCL2 and IRS1 and KRAS found in this analysis, were also downregulated (see Figure 3F).

There were 70 genes upregulated in PTC and downregulated in HT, while 31 genes were downregulated in PTC and upregulated in HT (see Figure 3G). The number of differential genes with opposite expression trends was far smaller than those with consistent trends. Therefore, we combined them for analysis. KEGG clustering analysis was performed on these 101 genes with opposite expression trends in PTC and HT. The clustered pathways for genes with opposite expression trends included ECM-receptor interaction and TNF signaling pathway, as well as some metabolic pathways, and pathways related to cell phagocytosis and connections (see Figure 3H). After drawing the pathway-gene diagram, two groups of genes were noteworthy (see Figure 3I). One group, located in the α circle in Figure 3H, includes the pro-proliferative kinase RPS6KA5, inflammation signal-related ubiquitin ligases CYLD and CBLB, and immune-related transcription factors NFATC1 and NFATC3. These were downregulated in PTC but upregulated in HT. The other group, located in β , mainly consists of extracellular matrix-related genes, including FN1, LAMC1, and LAMB1, and the cell adhesion protein DAG1. These were upregulated in PTC but downregulated in HT. Therefore, the differences between PTC and HT may primarily focus on some key cell proliferation pathways, ubiquitination status, and differences in extracellular matrix composition.

WGCNA Analysis:

After completing the differential gene analysis, we performed WGCNA analysis on all samples, corresponding to B in the data analysis flowchart, completing the analysis of Pathway4, Pathway5, and Pathway6. The samples were divided into 4 groups, and after WGCNA analysis (see Figure 4A), we found that the color block positively correlated (PosCorr) with both PTC and HT was red. The correlation coefficient of red with PTC was 0.26 ($P = 6 \times 10^{-4}$), and with HT was 0.22 ($P = 0.006$). The color block negatively correlated (NegCorr) with both PTC and HT was yellow, with a correlation coefficient of -0.33 ($P = 1 \times 10^{-5}$) with PTC and -0.17 ($P = 0.03$) with HT. The modules positively correlated with PTC and negatively correlated with HT were brown and magenta. In the brown module, the positive correlation coefficient with PTC was 0.69 ($P = 4 \times 10^{-25}$), and the negative correlation coefficient with HT was -0.18 ($P = 0.02$). In the magenta module, the positive correlation coefficient with PTC was 0.68 ($P = 5 \times 10^{-24}$), and the negative correlation coefficient with HT was -0.36 ($P = 1 \times 10^{-6}$). There were no modules negatively correlated with PTC and positively correlated with HT. The genes contained in each module are as follows: 108 genes in red, 292 genes in yellow, and 550 genes in brown and magenta combined.

After performing KEGG clustering and pathway-gene mapping on the red genes (Figures 4B and C), we also found that pathways such as p53 and apoptosis were highly expressed in both PTC and HT. Unlike the differential gene (DEG) analysis, in WGCNA we also found that the expression of DNA repair pathway genes was positively correlated with both diseases. Screening for HubGenes in the red module revealed that these disease-positively correlated HubGenes may have relatively close interactions between their encoded proteins (Figure 4D). They are mainly: DNA replication and repair-related genes (DNMT1, EZH2, CDK7, GINS1), cell cycle and chromosome regulation genes (CDC20, BUB1B, FOXM1, CDK1, etc.).

After performing KEGG clustering and pathway-gene mapping on the yellow genes (Figures 4E and F), similar to the differential gene analysis, we found that metabolism-related pathways were negatively correlated with PTC and HT. We also discovered genes related to neurological diseases such as Parkinson's and Alzheimer's. The HubGenes in the yellow module were not closely connected (Figure 4G). They are mainly protein modification and degradation-related genes (SUMO1, TSG10, USP46), cytoskeleton and signal transduction-related genes (ANKMY2). Protein modification and degradation-related genes play important roles in the pathogenesis of neurological diseases.

After performing KEGG clustering and pathway-gene mapping on the brown and magenta genes (Figures 4H and I), the genes were mainly clustered in pathways such as Focal adhesion, Regulation of actin cytoskeleton, Estrogen signaling pathway, and ECM-receptor interaction. Consistent with DEGs analysis, many cancer pathways were also enriched pathways for module genes. Similarly,

the HubGenes in these two color modules were not closely related (Figure 4J). They are mainly signal transduction and gene regulation-related genes (such as DUSP6, EFR3A, LRP4, etc.) and cell adhesion, structure, and interaction-related genes (SDC4, KRT19, and ITGA3).

Joint analysis of Differential Genes (DEGs) and WGCNA HubGenes:

To gain a more detailed understanding of the differences between PTC and HT, we combined the genes with opposite expression trends in DEGs with the HubGenes from the brown and magenta modules in WGCNA for PPI analysis, Figure 5A. 14 genes showed interactions, mainly involved in ECM-receptor interaction. We also plotted expression bar graphs for 12 of the more important genes (Figure 5C). The differences between PTC and HT may involve different extracellular matrices transmitting different signals to thyroid cells.

RT-qPCR and WB validation of clinical samples.

Using PTC, HT, and PTC para-carcinoma tissues we collected, we validated key genes obtained from the GEO data reanalysis using RT-qPCR and WB. These include TP53, which is upregulated in both PTC and HT, FN1, which is expressed oppositely in PTC and HT, key hubGenes such as DUSP6 and ETV5 that may link extracellular matrix and immune regulation from the joint analysis of DEGs and WGCNA, and the key transcription factors NFATC1 and NFATC3 that they may regulate to stimulate T cell activation. Results are shown in Figure 6.

WB detection of protein expression was generally consistent with the results obtained from GEO data. p53 protein was elevated in both HT and PTC, while compared to the control (para-carcinoma tissue, PT), FN1 showed opposite expression trends in HT and PTC. Among the hubGenes, NFATC1 and NFATC3 also showed opposite expression trends in HT and PTC. Other hubGenes such as DUSP6 and ETV5 showed lower expression in HT than in PTC, but both were higher than the control. RT-qPCR results were similar to WB, but genes with very low expression levels showed large standard deviations and did not demonstrate statistical differences.

Discussion

Thyroid cancer refers to malignant tumors originating from thyroid tissue and is one of the most common malignant tumors of the endocrine system. The incidence rate has been increasing annually, with a global average annual growth of 3-5% over the past 40 years. In 2020, the global age-standardized incidence rate was 11.6 per 100,000 for women and 3.5 per 100,000 for men, with women having a three-fold higher risk than men^{8,9}. Thyroid cancer mainly affects the follicular cells in the thyroid. Several common types of thyroid cancer are^{10,11}: Papillary Thyroid Carcinoma (PTC), accounting for about 80-85% of all thyroid cancer cases. This cancer mainly affects the follicular cells in the thyroid, usually grows slowly, and has a good prognosis. Follicular Thyroid Carcinoma (FTC) ac-

counts for 10-15% of thyroid cancer cases and also mainly affects follicular cells. It is slightly more invasive than papillary thyroid cancer, but the prognosis is still relatively good. Medullary Thyroid Carcinoma (MTC) accounts for 3-4% of thyroid cancer cases. This type of thyroid cancer originates from C cells (also called parafollicular cells) in the thyroid, which are responsible for secreting calcitonin. Anaplastic Thyroid Carcinoma (ATC) accounts for 1-2% of all thyroid cancer cases. Anaplastic thyroid cancer can originate from follicular cells, has a high degree of cellular variation, is highly invasive, and has a poor prognosis. Autoimmune Thyroid Diseases (AITD) are a group of organ-specific autoimmune diseases caused by the interaction between genetic susceptibility and environmental trigger factors. The most common among these are Hashimoto's disease and Graves' disease. Hashimoto's thyroiditis (HT), also known as chronic autoimmune thyroiditis and autoimmune hypothyroidism, has a global prevalence of 7.5%, with a higher incidence in women than in men (17.5% in women, 6% in men)¹². Cancer cells need to survive, so they must evade attacks from the immune system. The basis of autoimmune diseases is the immune system attacking its own tissues. The immune system's response to these two diseases seems completely opposite. But why do these two diseases coexist in thyroid cells? What are the similarities or differences in their molecular mechanisms? This study integrated data from four GEO databases, removed batch differences, and compared the expression data of thyroid cells from the main thyroid cancer: Papillary Thyroid Carcinoma (PTC), the main autoimmune thyroid disease: Hashimoto's Thyroiditis (HT), simultaneous PTC and HT (PTC_HT), and normal tissue (Control). The aim is to find the mechanism of coexistence of cancer and autoimmune diseases in thyroid cells.

We found that compared to normal tissue (Control), 202 genes showed differential expression in PTC, HT, and PTC_HT. Compared to Control, these 202 genes were mostly upregulated (Figure 2E), and the direction of expression increase and decrease was consistent in the three disease cell types studied (see Appendix 1). Most of these 202 genes are immune-related genes, clustered in pathways related to viral infection and autoimmune diseases. Inflammation may play a decisive role in the tumor microenvironment, and chemokines play an important role in the formation of peritumoral inflammation¹³. We also observed upregulation of chemokines CXCL8, CXCL9, CXCL10, and pro-inflammatory factor IL8 in all three diseases (Figure 2F, Appendix 1). It has been reported that abnormal thyroid-stimulating hormone receptor signaling increases the secretion of VEGF-A and CXCL8 by thyroid cancer cells, promoting angiogenesis and tumor growth. Inflammation and high levels of cytokines and chemokines seem to promote RET/PTC rearrangement, which is one of the key mutations in the process of thyroid cell carcinogenesis¹⁴. HT may be caused by the interaction of genetic and environmental factors¹⁵. Changes in iodine intake may be related to the occurrence of autoimmune thyroid diseases^{15,16}. The etiology of HT is still unclear. There is no definitive evidence on whether environmental factors, such

as iodine intake, and genetic factors disturb thyroid hormone receptor signaling, increase chemokine secretion, and create a chronic inflammatory environment leading to the formation of HT. However, the inflammatory environment caused by HT is likely to increase the occurrence of PTC^{17,18}. In summary, it can be seen that thyroid cells in PTC, HT, or PTC-HT are all in an environment where chemokines activate inflammation.

In addition to the upregulation of pro-inflammatory genes in the three diseases, we also observed upregulation of gene expression in the classic cancer-related p53 pathway (Figure 2F, Supplementary Table 1). For example, p53 and its target genes CDKN1A (p21) and PMAIP1 were all upregulated. p53 is a well-known tumor suppressor gene, referred to as the “guardian of the genome”¹⁹. When cells are subjected to stresses such as DNA damage and oxidative stress, p53 is activated and activates various downstream genes. For instance, p21 protein (CDKN1A) exerts anti-cancer effects by arresting the cell cycle. p53-mediated PMAIP1 activation can promote cell apoptosis²⁰. However, in the three aforementioned diseases, their expression was observed to be elevated. It appears that in the thyroid, the increase in p53 did not fulfill its expected function of inhibiting cell growth. In other thyroid cancers, such as anaplastic thyroid cancer (ATC), increased p53 protein levels have also been observed²¹. PMAIP1 has also been observed to have increased expression in follicular thyroid cancer (FTC)²². Two other important genes related to apoptosis and autoimmune diseases, FAS and BID, also showed significant upregulation (Figure 2F, G). FAS is a receptor that induces cell apoptosis, while BID is a pro-apoptotic protein. FAS acts as a protector against autoimmune diseases and tumor development by amplifying apoptosis of tumor cells or overactive immune cells through the activation of BID protein by hydrolysis²³. The above two anti-tumor mechanisms seem to have lost their function in PTC, HT, or PTC-HT. Their highly expressed pro-apoptotic effects were all blocked. We investigated downstream signaling molecules in the p53 and FAS pathways and found that the expression of anti-apoptotic proteins BCL2 and BCL2L2 genes was reduced (Figure 2G). These two proteins receive apoptotic signals from cells and form channels on the mitochondrial membrane to promote apoptosis²⁴. Whether the decrease in their expression is related to the “apoptosis resistance” of PTC or HT, and whether upregulation of their expression could be used to treat PTC or HT, deserves further study.

When comparing only the differentially expressed genes common to PTC and HT, we found that the phenomena of “chemokine-induced inflammatory environment” and “apoptosis resistance” in thyroid cells became more apparent. For genes upregulated in both diseases (Figure 3A-C), not only were key genes in the chemokine signaling pathway and apoptosis upregulated, but many genes in the Toll-like receptor signaling pathway and Th1, Th2, and Th17 cell differentiation pathways were also upregulated. In the comparison between PTC and HT, more chemokines and inflammatory factors were found to be overexpressed, such as chemokines 1, 5, 6, 11, various interleukin factors, and TNF-related pro-

teins. WGCNA analysis results (Figure 3) showed that genes in the red module (positively correlated gene module for PTC and HT) also exhibited upregulation of p53 and apoptosis-related pathway genes (Figure 4). After clustering analysis of genes downregulated in both diseases (Figure 3D-F), it was found that in addition to BCL2, ATG5, a gene resistant to autophagy, was also downregulated. ATG5 is used to eliminate damaged organelles, and its complex helps extend and close autophagosome membranes. Without sufficient ATG5 protein, the formation of autophagosomes would be hindered²⁵. The lack of ATG5 may also lead to chronic inflammation²⁶. Whether this is the reason for the formation of the cellular microenvironment in PTC and HT is worth in-depth study. In addition, genes downregulated in both PTC and HT were also clustered in multiple metabolic pathways, similar to the results of gene clustering analysis in the yellow module (negatively correlated gene module for PTC and HT) using WGCNA analysis (Figure 3). Genes in sugar, lipid, and amino acid metabolic pathways were downregulated in both diseases. Thyroid cells in PTC and HT may have similar metabolic patterns.

In addition to focusing on the consistent features of PTC and HT, we also investigated the differentially expressed genes that were different between PTC and HT. Since the 202 common differentially expressed genes among PTC, HT, and PTC_HT (Figure 2D) showed consistent directions of increase and decrease compared to normal tissues, we only used PTC and HT compared to the control group separately, and set $|\text{LogFC}| > 0$ and $\text{Padj} < 0.05$ as conditions to screen for differentially expressed genes and perform cluster analysis. There were 70 genes upregulated in PTC and downregulated in HT, and 31 genes downregulated in PTC and upregulated in HT (Figure 3G). After clustering analysis of these 101 genes, we found that the differentially expressed genes were mainly concentrated in MAPK, TNF, parathyroid hormone, and T cell receptor signaling pathways (α region in Figure 3I). Secondly, they were also concentrated in the ECM-receptor interaction pathway (β region in Figure 3I). In PTC, multiple signal pathway-related genes (NFATC1, NFATC3, CYLD, CBLB, and RPS6KA5) were downregulated, while ECM receptor-related genes (FN1, LAMB1, LAMC1, and DAG1) were upregulated. The expression of these genes was opposite in HT (see Figure 5C). By selecting hub genes from modules representing opposite correlations with PTC and HT (brown and magenta) in the WGCNA analysis and performing String analysis, we found that four hub genes: DUSP6, ETV4, ETV5, and SDC4 may have potential interactions. More surprisingly, these four genes could connect the differentially expressed genes in MAPK, TNF, parathyroid hormone, and T cell receptor signaling pathways with those in the ECM-receptor interaction pathway discovered by DEG analysis, as shown in the String analysis Figure 5A. We speculate that the difference between PTC and HT may be reflected in changes in the extracellular matrix. In cancer, the upregulation of extracellular matrix-related genes (FN1, LAMB1, LAMC1, and DAG1) may downregulate transcription factors NFATC1 and NFATC3 through the SDC4-DUSP6-ETV5

axis, thereby altering the proliferation status of cells and causing cancer cell transformation. In HT patient cells, matrix-related genes are downregulated. The increased expression of NFATC1 and NFATC3 prevents cancer transformation. Using spatial transcriptomics, Kai Yan et al. discovered key ligand-receptor interactions, including LAMB3-ITGA2, FN1-ITGA3, and FN1-SDC4, involved in the process of PTC development, where cells transform from typical follicular cells (FC) to atypical follicular cells (AFC), and finally to tumor cells, reducing patient survival rates²⁷. The role of FN1-SDC4 may also promote the response of pancreatic stellate cells (PSCs) to tumor cell signals²⁸. NFATC1 and NFATC3 are transcription factors and important downstream factors in the cellular calcium signaling pathway, widely involved in immune regulation, development, and multiple physiological processes of growth. NFATC1 and NFATC3 are key factors activated in T cells after antigen stimulation^{29,30}. They are also associated with the occurrence of autoimmune diseases^{31,32}. We also observed upregulation of NFATC1 and NFATC3 expression in HT, while their expression was downregulated in PTC. In summary, we speculate that the molecular expression differences between PTC and HT mainly focus on the differential expression of extracellular matrix, which activates/inhibits T cell immune responses, leading to different disease phenotypes.

Of course, the interrelationships between these genes in our hypothesis are not yet fully understood, but the results of this study suggest that future research could pay more attention to these different pathogenesis mechanisms of cancer and autoimmune diseases that can occur simultaneously in thyroid cells. This provides new directions for understanding the changes in thyroid cells and their microenvironment in relation to these two diseases, and for their treatment.

Conclusion

Based on the analysis of this study, we believe that: Both papillary thyroid cancer (PTC) and Hashimoto's thyroiditis (HT) are in a state of chronic inflammation and do not respond to p53 and FAS-induced apoptosis signals. There are significant differences between the two in extracellular matrix and immune response regulation, which may lead to different disease phenotypes. This provides a new perspective for the mechanism research and treatment of the two diseases.

Authors' contributions

Jie Yang contributed to the study concept and design; Wenxia Wang participated in the sample collection; Weibin Fan contributed to data management, Yuqing Ma performed Western blot and cell culture; Zhizheng Xu contributed to data analysis; Xuelu Xu contributed to the manuscript and figures.

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Not applicable.

Declarations

Conflict of Interests/Competing Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Figures And Legends:

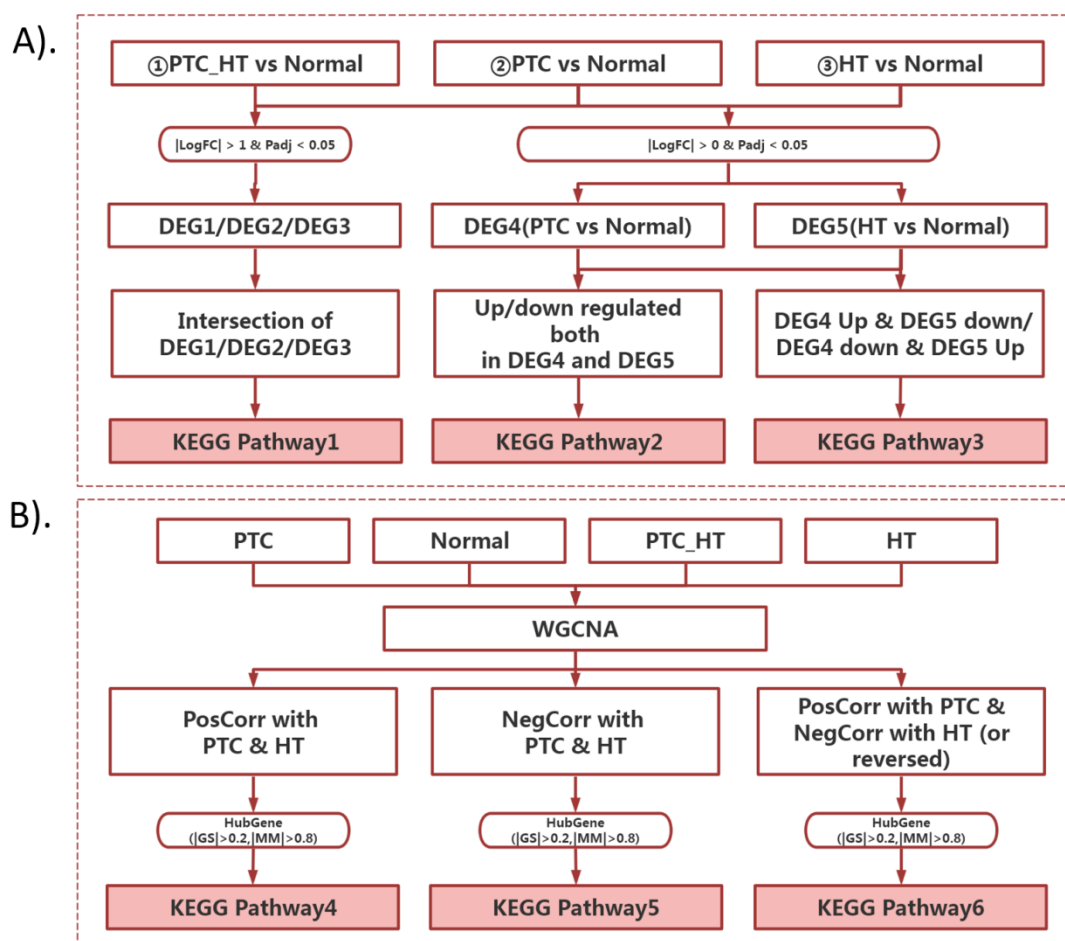


Figure 1. Data analysis flowchart. A) Differential gene analysis and clustering of SVA-corrected data using the limma package. B) Analysis of control (Normal), PTC, HT, and PTC_HT using the WGCNA method and pathway clustering analysis based on the results.

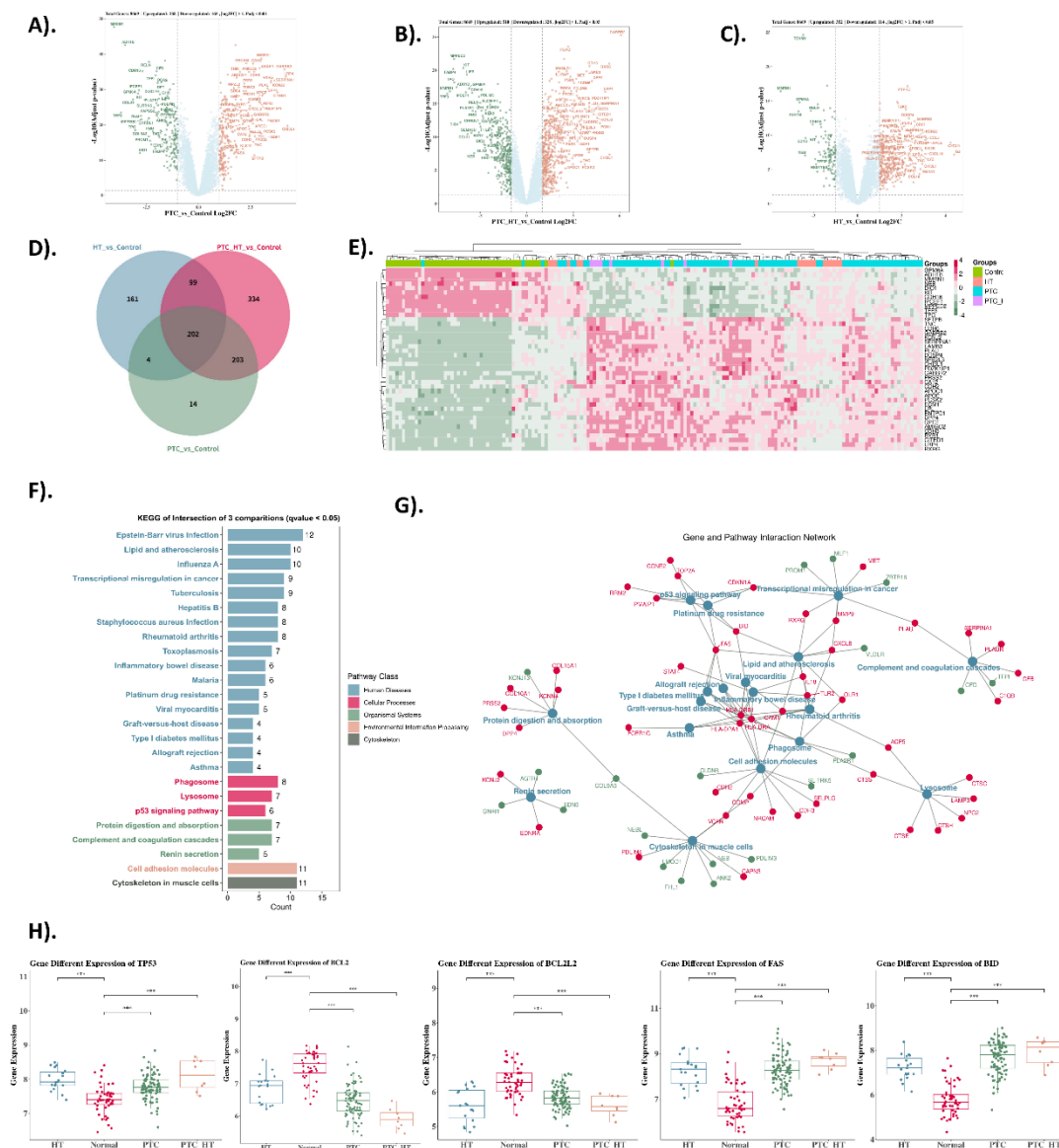


Figure 2. Differential gene expression analysis among PTC_HT, PTC, HT, and control (Control) groups. Figures A, B, and C show volcano plots of PTC_HT vs. Control (510 upregulated, 328 downregulated), PTC vs. Control (258 upregulated, 165 downregulated), and HT vs. Control (352 upregulated, 114 downregulated), respectively. Figure D: Venn diagram of differentially expressed genes, showing 202 common differentially expressed genes among the three diseases. Figure E: Heatmap displaying the relationships between four tissues, drawn with the top 50 differentially expressed genes. Figures F and G: KEGG analysis and pathway-gene relationship diagram of the 202 genes, revealing gene changes related to infection, complement, and p53 pathways. Figure H: Showing that TP53 is upregulated in all three diseases, while its downstream anti-apoptotic genes BCL2 and BCL2L2 are downregulated, suggesting that activation of this apoptotic signaling pathway fails to effectively inhibit the occurrence of cancer or autoimmune diseases.

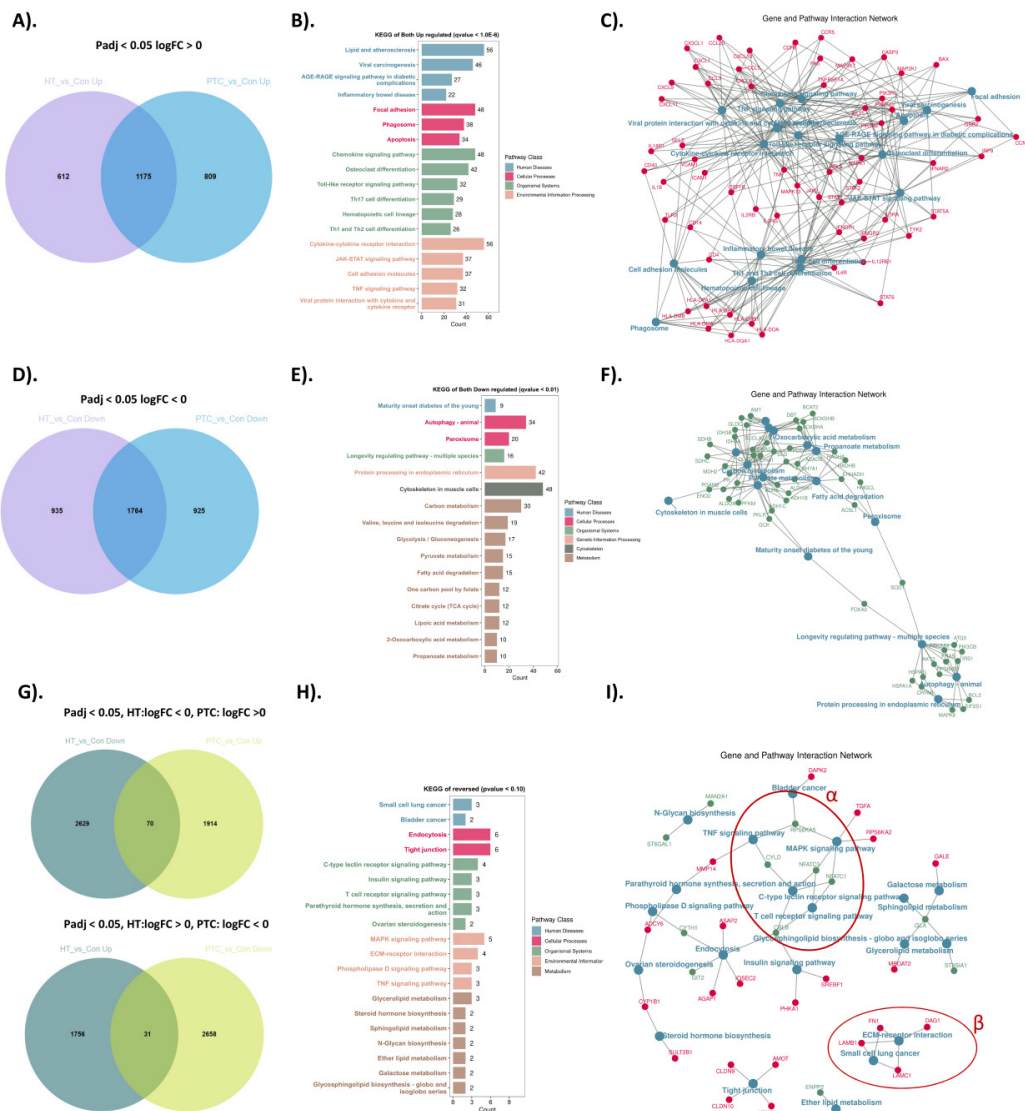


Figure 3. Analysis of common differential genes in PTC and HT. A and B: The 1175 upregulated genes common to PTC and HT, with clustering analysis showing they are mainly concentrated in immune regulation and apoptosis-related pathways. C: The pathway-gene diagram displays a large number of chemokines (such as CXCL8, CXCL9, CXCL10) and pro-inflammatory factors (such as CD4, IL8) with upregulated expression. D and E: The 1764 downregulated genes common to PTC and HT, showing they are mainly concentrated in metabolism-related pathways, such as carbon, glucose, and amino acid metabolism. F: Genes downregulated in PTC and HT, including apoptosis-related genes such as BCL2, IRS1, and KRAS also showed decreased expression. G: Genes with opposite expression trends in PTC and HT, with 70 genes upregulated in PTC and downregulated in HT, and 31 genes downregulated in PTC and upregulated in HT. H: KEGG clustering analysis showed that the differential genes with opposite expression trends mainly focus on multiple pathways such as ECM-receptor interaction and the TNF signaling pathway. I: The pathway-gene diagram shows that among the genes with opposite expression trends, RPS6KA5, CYLD, CBLB, NFATC1, and NFATC3 are downregulated in PTC but upregulated in HT; while FN1, LAMC1, LAMB1, and DAG1 are upregulated in PTC but downregulated in HT. Note: In the pathway-gene diagram, red represents upregulation, and green represents downregulation.

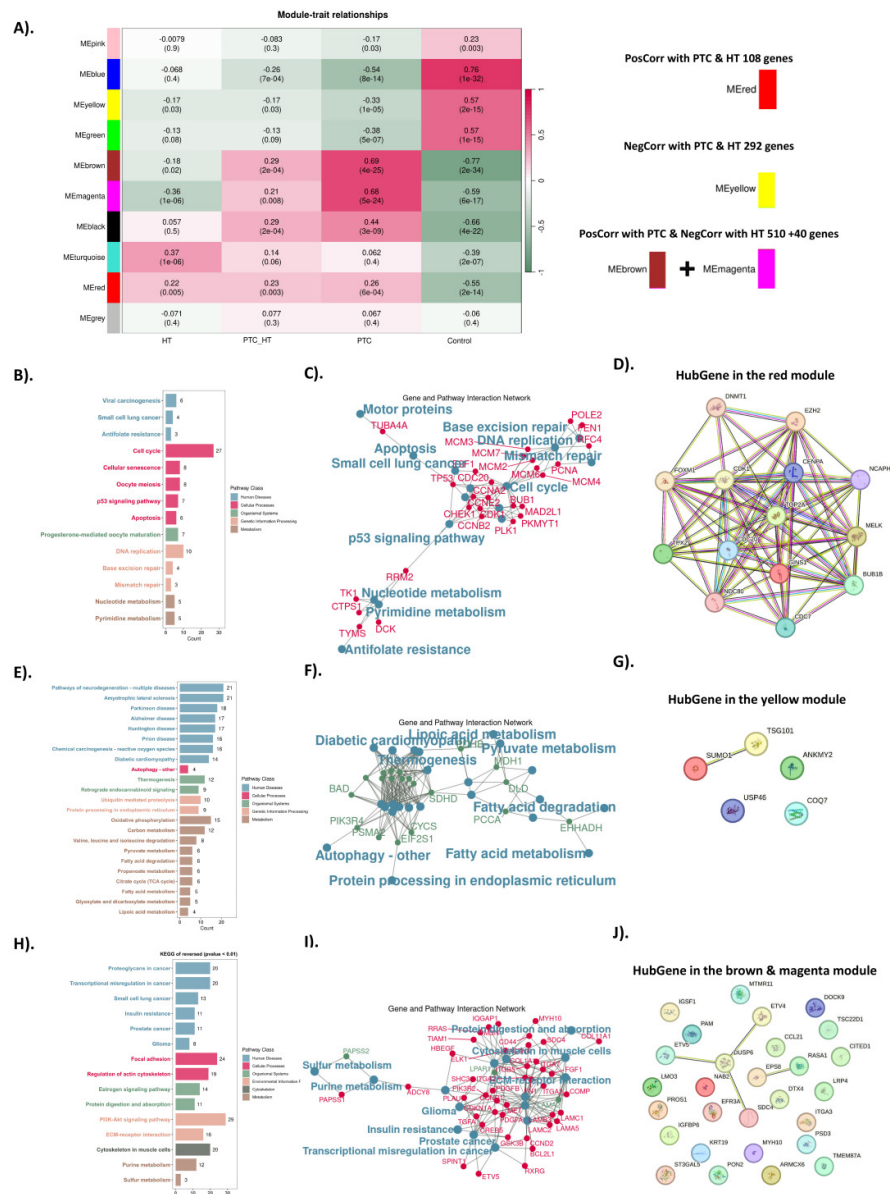


Figure 4. WGCNA analysis results. A: Module identification results of WGCNA analysis after dividing samples into four groups. The red module is positively correlated with both PTC and HT; the yellow module is negatively correlated with both PTC and HT; the brown and magenta modules are positively correlated with PTC and negatively correlated with HT. B, C: KEGG clustering analysis and pathway-gene diagram of genes in the red module. High expression of p53 and apoptosis-related pathways and DNA repair pathway genes are found in both PTC and HT. D: Interaction network of Hub genes in the red module, mainly including genes related to DNA replication and repair and genes regulating the cell cycle and chromosomes. E, F: KEGG clustering analysis and pathway-gene diagram of genes in the yellow module. Metabolism-related pathways are negatively correlated with PTC and HT, and also involve genes related to neurological diseases. G: Interaction network of Hub genes in the yellow module, mainly including genes related to protein modification and degradation (SUMO1, TSG101, USP46) and genes related to the cytoskeleton and signal transduction (ANKMY2). H, I: KEGG clustering analysis and pathway-gene diagram of genes in the brown and magenta modules. J: Interaction network of Hub genes in the brown and magenta modules.

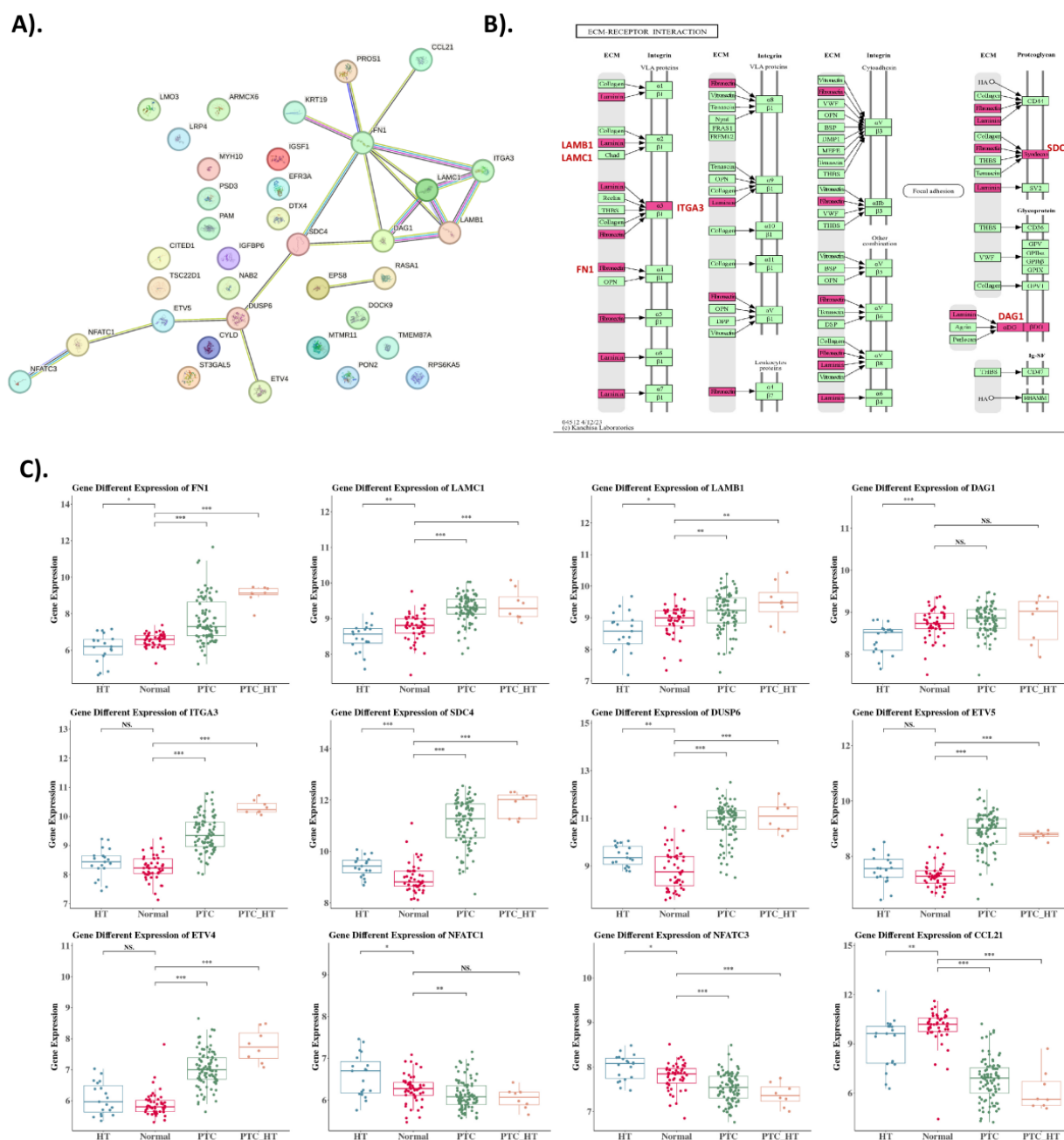


Figure 5. Combined analysis of DEGs and Hub Genes. A: Protein-protein interaction (PPI) analysis of genes with opposite expression trends in DEGs and Hub Genes in the brown and magenta modules of WGCNA. B: KEGG pathway diagram of extracellular matrix gene expression. C: Bar chart of the expression of 12 important genes found in the related gene network in PPI analysis.

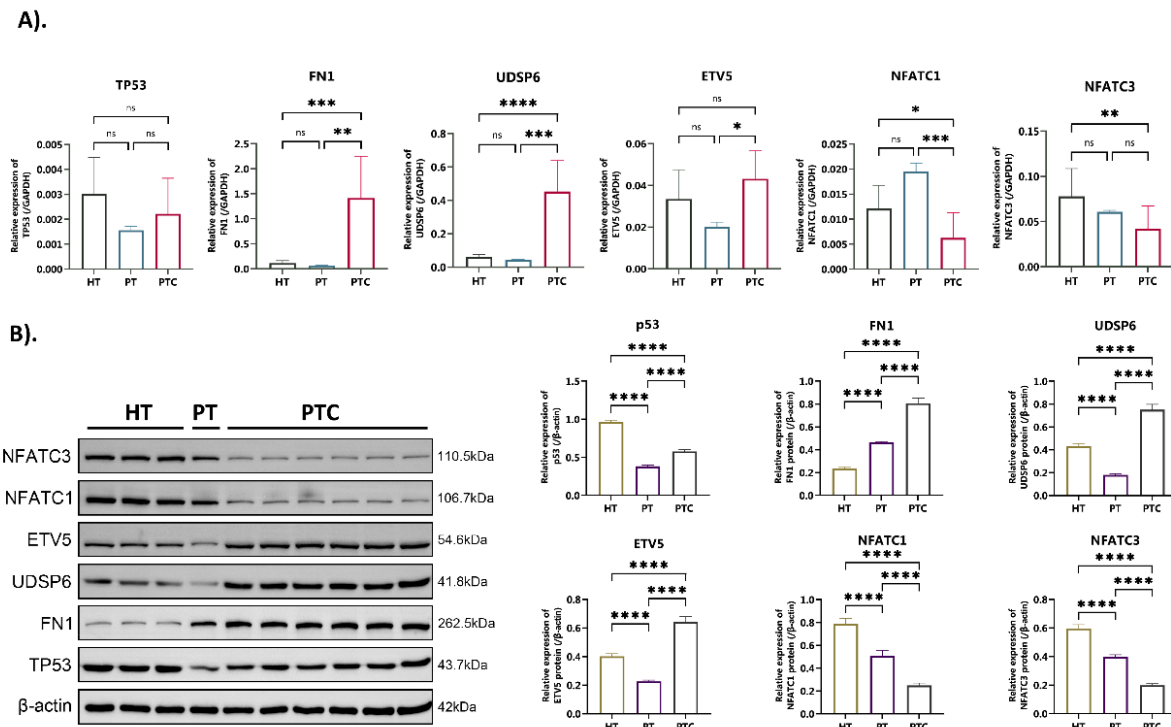


Figure 6. Expression of DEGs and Hub Genes in different thyroid tissues. HT: Hashimoto's Thyroiditis (n = 3), PT: Peritumoral tissue (n = 1 Control), PTC: Papillary Thyroid Carcinoma (n = 6). A: RT-qPCR results of 6 genes, with each sample repeated three times. B: Western Blotting results of 6 genes, with each sample repeated three times.

References

1. Xue X, Wu D, Yao H, Wang K, Liu Z, Qu H. Mechanisms underlying the promotion of papillary thyroid carcinoma occurrence and progression by Hashimoto's thyroiditis. *Front Endocrinol (Lausanne)* 2025; 16:1551271.
2. Zheng K, Mao J. Comparison and Analysis of Clinical Features of Papillary Thyroid Cancer Complicated With Hashimoto's Thyroiditis. *Clin Med Insights Oncol* 2024; 18:11795549241287085.
3. Du X, Chen W. Bioinformatic analysis of *serpin1* expression in papillary thyroid carcinoma and its potential association with Hashimoto's thyroiditis. *Discov Oncol* 2025; 16 (1):356.
4. Sulaieva O, Chernenko O, Selesnov O, Nechay O, Maievskiy O, Falalyeyeva T, et al. Mechanisms of the Impact of Hashimoto Thyroiditis on Papillary Thyroid Carcinoma Progression: Relationship with the Tumor Immune Microenvironment. *Endocrinol Metab (Seoul)* 2020; 35 (2):443-455.
5. Li B, Cai Z, Zhang Y, Chen R, Tang S, Kong F, et al. Biomarkers associated with papillary thyroid carcinoma and Hashimoto's thyroiditis: Bioinformatic analysis and experimental validation. *Int Immunopharmacol* 2024; 143 (Pt 3):113532.
6. Kim SJ, Lee SE, Kim YI, Nam-Goong IS, Jung HW, Kim ES. Papillary thyroid cancer with Hashimoto's thyroiditis attenuates the tumour aggressiveness through the up-regulation of E-cadherin and TGF- β expression. *Clin Exp Med* 2023; 23 (3):833-840.
7. Leek JT, Johnson WE, Parker HS, Jaffe AE, Storey JD. The *sva* package for removing batch effects and other unwanted variation in high-throughput experiments. *Bioinformatics* 2012; 28 (6):882-883.
8. Almansoori A, Busch H, Bendardaf R, Hamoudi R. Thyroid cancer incidence in the United Arab Emirates: a retrospective study on association with age and gender. *F1000Res* 2022; 11:338.

9. Lim H, Devesa SS, Sosa JA, Check D, Kitahara CM. Trends in Thyroid Cancer Incidence and Mortality in the United States, 1974-2013. *JAMA* 2017; 317 (13):1338-1348.
10. Boucai L, Zafereo M, Cabanillas ME. Thyroid Cancer: A Review. *JAMA* 2024; 331 (5):425-435.
11. Voelker R. What Is Thyroid Cancer? *JAMA* 2024; 332 (4):346.
12. Sapountzi E, Tsinopoulou VR, Kotanidou EP, Giza S, Galli-Tsinopoulou A. Coexistence of Autoimmune Thyroiditis and Juvenile Idiopathic Arthritis. *Cureus* 2023; 15 (8):e44384.
13. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature* 2008; 454 (7203):436-444.
14. Guarino V, Castellone MD, Avilla E, Melillo RM. Thyroid cancer and inflammation. *Mol Cell Endocrinol* 2010; 321 (1):94-102.
15. Ragusa F, Fallahi P, Elia G, Gonnella D, Paparo SR, Giusti C, et al. Hashimotos' thyroiditis: Epidemiology, pathogenesis, clinic and therapy. *Best Pract Res Clin Endocrinol Metab* 2019; 33 (6):101367.
16. Camargo RY, Tomimori EK, Neves SC, I GSR, Galrao AL, Knobel M, et al. Thyroid and the environment: exposure to excessive nutritional iodine increases the prevalence of thyroid disorders in Sao Paulo, Brazil. *Eur J Endocrinol* 2008; 159 (3):293-299.
17. Dos Santos Valsecchi VA, Betoni FR, Ward LS, Cunha LL. Clinical and molecular impact of concurrent thyroid autoimmune disease and thyroid cancer: From the bench to bedside. *Reviews in Endocrine & Metabolic Disorders* 2024; 25 (1):5-17.
18. Ferrari SM, Fallahi P, Elia G, Ragusa F, Ruffilli I, Paparo SR, et al. Thyroid autoimmune disorders and cancer. *Semin Cancer Biol* 2020; 64:135-146.
19. Engeland K. Cell cycle regulation: p53-p21-RB signaling. *Cell Death Differ* 2022; 29 (5):946-960.
20. Gao Y, Huang A, Zhao Y, Du Y. PMAIP1 regulates autophagy in osteoblasts via the AMPK/mTOR pathway in osteoporosis. *Hum Cell* 2024; 37 (4):1024-1038.
21. Lam KY, Lo CY, Chan KW, Wan KY. Insular and anaplastic carcinoma of the thyroid: a 45-year comparative study at a single institution and a review of the significance of p53 and p21. *Ann Surg* 2000; 231 (3):329-338.
22. Wang H, Shang F, Wang Y, Pang B, Kang L, Zhou C, et al. PMAIP1 regulates the progression of follicular thyroid carcinoma through the Wnt3/FOSL1 pathway. *Front Oncol* 2025; 15:1502391.
23. Strasser A, Jost PJ, Nagata S. The many roles of FAS receptor signaling in the immune system. *Immunity* 2009; 30 (2):180-192.
24. Deng J. How to unleash mitochondrial apoptotic blockades to kill cancers? *Acta Pharm Sin B* 2017; 7 (1):18-26.
25. Codogno P, Meijer AJ. Atg5: more than an autophagy factor. *Nat Cell Biol* 2006; 8 (10):1045-1047.
26. Gukovsky I, Gukovskaya AS. Impaired autophagy triggers chronic pancreatitis: lessons from pancreas-specific atg5 knockout mice. *Gastroenterology* 2015; 148 (3):501-505.
27. Yan K, Liu QZ, Huang RR, Jiang YH, Bian ZH, Li SJ, et al. Spatial transcriptomics reveals prognosis-associated cellular heterogeneity in the papillary thyroid carcinoma microenvironment. *Clin Transl Med* 2024; 14 (3):e1594.
28. Zourelidis A, Trojanowicz B, Sunami Y, Hause G, Vieweg D, Kleeff J. Distance-depending transcriptome changes of pancreatic stellate cells in paracrine pancreatic ductal adenocarcinoma co-culture models. *Sci Rep* 2024; 14 (1):18030.
29. Peng SL, Gerth AJ, Ranger AM, Glimcher LH. NFATc1 and NFATc2 together control both T and B cell activation and differentiation. *Immunity* 2001; 14 (1):13-20.
30. Urso K, Alfranca A, Martinez-Martinez S, Escolano A, Ortega I, Rodriguez A, et al. NFATc3 regulates the transcription of genes involved in T-cell activation and angiogenesis. *Blood* 2011; 118 (3):795-803.
31. Sheak JR, Jones DT, Lantz BJ, Maston LD, Vigil D, Resta TC, et al. NFATc3 regulation of collagen V expression contributes to cellular immunity to collagen type V and hypoxic pulmonary hypertension. *Am J Physiol Lung Cell Mol Physiol* 2020; 319 (6):L968-L980.
32. Xiao Y, Qureischi M, Dietz L, Vaeth M, Vallabhapurapu SD, Klein-Hessling S, et al. Lack of NFATc1 SUMOylation prevents autoimmunity and alloreactivity. *J Exp Med* 2021; 218 (1).