

Downregulation of CDH16 in Papillary Thyroid Cancer and Its Potential Molecular Mechanism Analysed by qRT-PCR, TCGA and in silico Analysis

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Objective: Thyroid cancer has the highest prevalence among the cancer types that affect the endocrine system; however, its molecular mechanisms are not yet determined. Cadherin-16 (CDH16) plays an important role in the tumorigenesis of human cancers, but its influence on papillary thyroid cancer (PTC) is poorly investigated. This study aimed to explore the role of CDH16 in PTC.

Methods: We performed quantitative real-time polymerase chain reaction to investigate CDH16 expression in PTC. The clinical significance of CDH16 expression in PTC was then evaluated using The Cancer Genome Atlas (TCGA) database. Bioinformatics analysis was also conducted to determine the potential molecular mechanisms of CDH16.

Results: CDH16 was remarkably downregulated in PTC tumors compared with that in corresponding normal thyroid tissues in the local and TCGA cohorts. This downregulation was associated with unfavorable clinicopathological features, including histological type, high tumor stage, aggressive lymph node metastasis (LNM), and advanced clinical stage. In addition, logistic analyses revealed that the reduced expression of CDH16 can aggravate the risk of LNM in PTC. Bioinformatics analysis indicated that the co-expressed CDH16 genes mainly participated in signaling the cancer-related pathways.

Conclusion: CDH16 is involved in PTC progression and acts as an LNM-related gene in PTC.

Keywords: CDH16, papillary thyroid cancer, lymph node metastasis, co-expression, mechanism

Introduction

Thyroid cancer has the highest prevalence among the cancers that affect the endocrine system worldwide. In 2017, approximately 56,870 cases of thyroid cancer were diagnosed in America, and approximately 2010 patients succumbed to the disease.¹ In China, approximately 90,000 diagnosis with thyroid cancer and 6800 deaths were recorded in 2015.² Papillary thyroid cancer (PTC) is the most common subtype of thyroid cancer and accounts for more than 80% of all the thyroid cancer cases.^{3,4}

PTC patients have relatively better prognosis compared with other cancer patients. After proper surgical treatments and radiotherapy, more than 90% of the patients with PTC can have a 10-year survival.⁵ However, some patients might suffer from disease recurrence and death even after timely treatments.⁶ Lymph node metastasis (LNM) is a potential factor that can aggravate the risk of locoregional

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recurrence and mortality in patients with PTC.^{7–10} Recent studies revealed that several molecular biomarkers, such as BRAF^{11,12} TERT,^{13–15} PIK3CA^{16,17} can facilitate LNM to some extent. Despite the in-depth research and progress on PTC studies, comprehensive clinical evaluation and molecular mechanistic exploration remain lacking. Therefore, searching for novel functional biomarkers in PTC might provide preliminary data and a fundamental theoretical basis for the elucidation of PTC tumorigenesis.

Cadherins (CDHs) belong to a superfamily of glycoproteins that plays critical roles in calcium-dependent cell-to-cell adhesion.^{18,19} CDHs also participate in embryonic development, normal cell function, and tissue integrity preservation via homotypic and homophilic interactions between extracellular regions.²⁰ Cadherin-16 (CDH16), a member of the CDH gene family and is localized to chromosome 16, might be involved in some certain diseases.^{21–25} However, studies on the clinical significance and regulatory mechanisms of CDH16 expression in PTC are required to verify this assumption. Further studies are necessary to identify the CDH16 expression in PTC, determine the regulatory mechanisms, and provide novel insights into PTC tumorigenesis.

In this study, we performed quantitative real-time polymerase chain reaction (qRT-PCR) to detect CDH16 expression in PTC tumors and corresponding normal thyroid tissues. The results were further validated using The Cancer Genome Atlas (TCGA) database. The relationship of CDH16 expression with clinicopathological features and LNM in PTC was also analyzed. Several bioinformatics analysis methods, including Gene Ontology (GO), Kyoto Encyclopedia of Gene and Genomes (KEGG) and protein–protein interaction (PPI), were performed to determine the potential molecular mechanisms of CDH16.

Materials and Methods

Clinical Samples

We collected 16 tissues of cases of clinical PTC tumors and corresponding noncancerous thyroid tissues during surgeries from February 2011 to June 2013 at the Department of Neck surgery of the Second Affiliated Hospital of Wenzhou Medical University. We used liquid nitrogen to snap-freeze these samples immediately after resection and then stored at -80°C for followed RNA isolation. Two senior pathologists were invited to review all the samples retrospectively and to confirm the histological diagnosis and to ensure abundant cancer content of

the tumor using the double-blind method. All participants signed informed consent for the scientific use of biological material. This research protocol was approved by the Ethics Committee of the Second Affiliated Hospital of Wenzhou Medical University.

Clinicopathological Feature of Local Patients with PTC

This study involved 16 patients, including three men (18.8%) and 13 women (81.2%) (male-to-female ratio = 0.231). The patients were 27–75 years of age at diagnose with a mean age of 54.1 years. Among them, eight patients suffered from LNM. No significant difference was observed between the local and TCGA cohorts due to the small case series of the former. Detailed clinicopathological data are shown in Table 1.

Detection of CDH16 Expression by Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

We used TRIzol reagent (Life Technologies, Carlsbad, CA) to extract total RNA from the paired PTC tumors and non-cancerous thyroid tissues according to the manufacturer's indication, and then the RNA purity and concentration were

Table 1 The Clinicopathologic Features in the Local Cohort and in the TCGA cohort

Clinicopathologic Features	Local (n=16) (%)	TCGA (n=505) (%)	P-Value
Age (year)			
Mean	54.06 ± 14.31	47.22 ± 15.83	0.089
>45	12 (75.0)	264 (52.3)	0.076
≤45	4 (25.0)	239 (47.3)	
Gender			0.665
Male	3 (18.8)	135 (26.7)	
Female	13 (71.2)	368 (72.9)	
Tumor size (mm)			0.17
>10	9 (56.2)	303 (60.0)	
≤10	7 (43.8)	102 (20.2)	
LNM			0.965
Yes	8 (50.0)	224 (44.4)	
No	8 (50.0)	229 (45.3)	
Multifocality			0.743
Yes	8 (50.0)	267 (52.9)	
No	8 (50.0)	226 (44.8)	
Clinical stage			0.149
I + II	8 (50.0)	337 (66.7)	
III + IV	8 (50.0)	164 (32.5)	

Abbreviation: LNM, lymph nodes metastasis.

measured by Plate reader Infinite 200 PRO (Tecan, Swiss). We used ReverTra Ace qPCR RT Kit (Toyobo, Osaka, Japan) for cDNA preparation. qRT-PCR analysis of CDH16 expression was performed in triplicate using the Thunderbird SYBR qPCR Mix (Toyobo, Osaka, Japan) in the Applied Biosystems 7500 Real-time PCR system (Applied biosystems, Foster City, CA). We set the expression of GAPDH as an internal control. The primer sequences of CDH16 were as follows: forward 5'- CCTCATCCTCATTTTCACC -3' and reverse 5'-GGGCTTCTACTCTGTCCTG -3'.

Retrieval of the Cancer Genome Atlas (TCGA) Data

We downloaded the clinical information and corresponding RNA-seq data of CDH16, including 505 PTC tumors and 59 normal thyroid tissues, from TCGA database.

Retrieval of CDH16 Co-Expression Genes' Data

To investigate the potential mechanisms of CDH16 expression in PTC tumors, we acquired CDH16-related co-expressed genes from the MEM and cBioPortal database. In MEM, the results of gene probe were extracted from MEM based on a P-value <0.0001. Pearson's correlation analysis was used in cBioPortal data to evaluate the correlation between CDH16 and co-expressed genes. The present research included the genes with an absolute value of the Pearson's correlation coefficient >0.4 in PTC. Then, the intersection results between MEM and cBioPortal were used for further study.

Bioinformatics Analyses

In this study, we used bioinformatics analyses to preliminarily discuss the intrinsic molecular mechanisms of CDH16 in PTC. The selected overlapping co-expressed

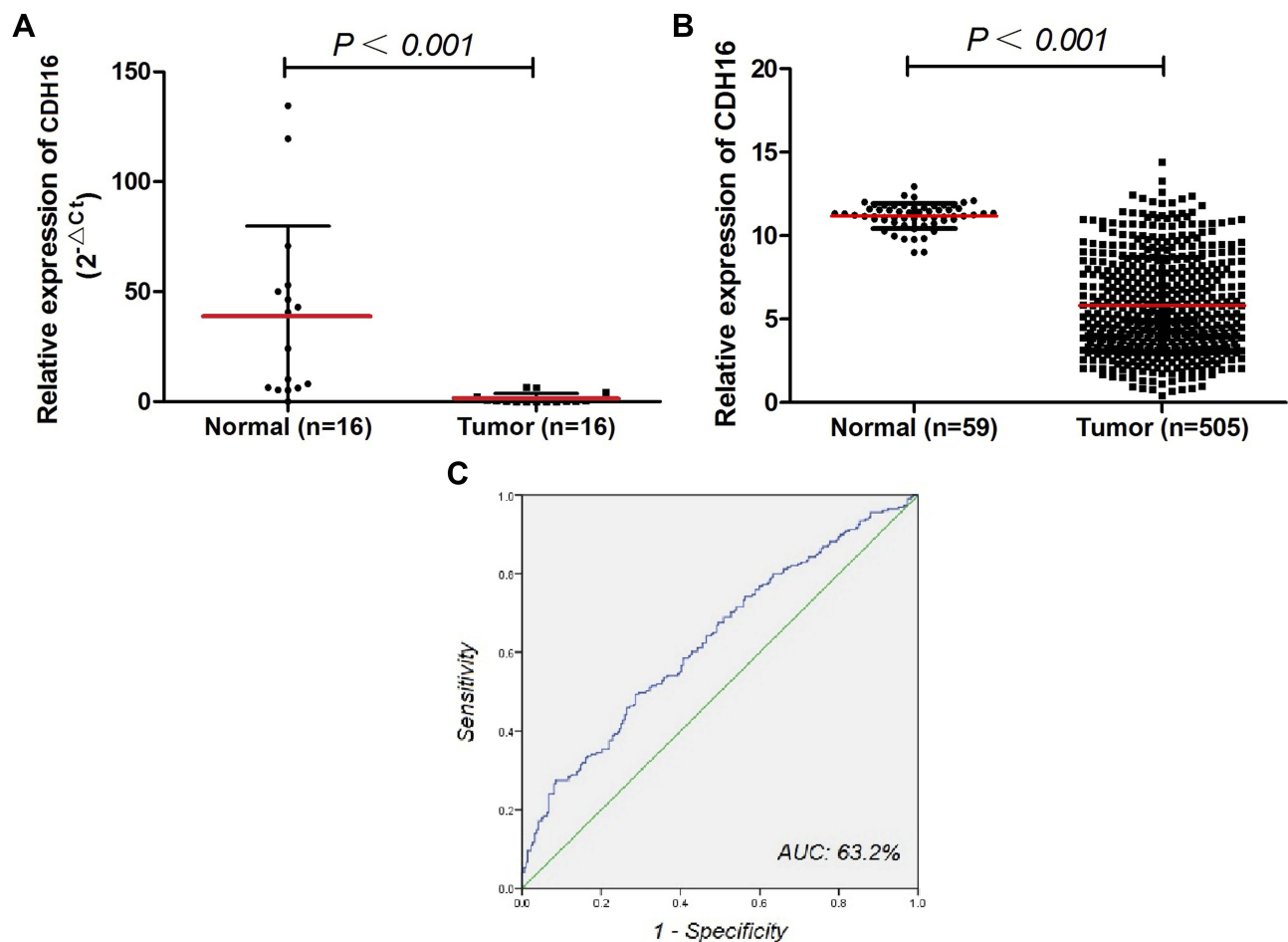


Figure 1 CDH16 expression was significantly downregulated in thyroid cancer samples compared with normal thyroid tissues in local and TCGA cohorts.

Notes: (A) In the local cohort, CDH16 expression was examined by qRT-PCR in 16 paired PTC samples and adjacent normal tissues (Mann–Whitney U-test, $P < 0.001$). (B) The TCGA cohort contained 505 tumor samples and 59 normal tissue samples. (C) ROC curve for CDH16 expression to diagnose PTC in the TCGA cohort.

genes in MEM and cBioPortal were used for GO and KEGG analyses in David v6.7 to elucidate the enrichment of genes in biological processes and signaling pathways. We also performed PPI analyses in STRING to explore proteins interaction association to hypothesize the potential pathways through which CDH16 participates in PTC tumorigenesis. Cytoscape 3.6.1 software was used to draw the functional networks.

Statistical Analyses

We used SPSS version 22.0 (IBM, New York, USA) to perform statistical analyses in the present study. The expression of CDH16 in PTC tumors and normal thyroid tissues detected by qRT-PCR were expressed as the means \pm SD, and the Mann–Whitney *U*-test was used to determine significance. We used Student's *t*-test to test the data on normal distribution. And chi-square test or Fisher's exact test was used to elucidate the relationship between clinicopathological factors and CDH16 expression. We performed logistic regression analyses to evaluate the lymph node metastatic risk of CDH16. All P-values were two-sided, and a P-value of <0.05 was considered statistically significant. GraphPad Prism 5 (GraphPad Software, La Jolla, CA, USA) was used for graphs.

Results

CDH16 Expression in Patients with PTC

We detected the mRNA expression of CDH16 in 16 pairs of PTC tumors and corresponding noncancerous thyroid tissues by qRT-PCR to confirm the role of CDH16 in PTC. As shown in [Figure 1A](#), CDH16 expression was significantly downregulated in the tumor samples compared with that in the corresponding noncancerous thyroid tissues ($P < 0.05$). This result was also validated by the TCGA database, which revealed that the CDH16 expression was lower in PTC samples than in normal thyroid tissues ([Figure 1B](#)). A receiver operator characteristic (ROC) curve was plotted to investigate the diagnostic value of CDH16. According to the TCGA database, 78.0% and 71.2% were the sensitivity and specificity rates, respectively, which can be used to distinguish the normal tissues from PTC tumors ([Figure 1C](#)).

Relationship Between CDH16 Expression and Clinicopathological Factors in PTC

We investigated the relationship between CDH16 expression and clinicopathological factors. The 505 patients with PTC from the TCGA database were divided into low ($n=252$) and high CDH16 expression ($n=253$) groups on

Table 2 The Relationship Between CDH16 Expression and Clinicopathologic Features in the TCGA Cohort

Clinicopathologic Features	Low Expression (n=252) (%)	High Expression (n=253) (%)	P-Value
Age (year)			
Mean	46.74 \pm 15.61	47.70 \pm 16.08	0.497
>45	133 (52.8)	131 (51.8)	0.750
\leq 45	117 (46.4)	122 (48.2)	
Gender			0.825
Male	66 (26.2)	69 (27.3)	
Female	184 (73.0)	184 (72.7)	
Histological type			< 0.001
Classical	196 (77.8)	160 (63.2)	
Other types	50 (19.8)	86 (34.0)	
Tumor size (mm)			
Mean	18.60 \pm 9.15	16.11 \pm 8.93	0.060
>10	164 (65.1)	139 (55.2)	0.036
\leq 10	43 (17.1)	59 (23.3)	
Multifocality			0.052
Yes	144 (57.1)	123 (48.6)	
No	102 (40.5)	124 (49.0)	
LNM			0.001
Yes	133 (52.8)	91 (36.0)	
No	99 (39.3)	130 (51.4)	
Tumor stage			0.001
I + II	135 (53.5)	174 (68.8)	
III + IV	114 (45.2)	78 (30.9)	
Clinical stage			0.029
I + II	156 (61.9)	181 (71.6)	
III + IV	93 (36.9)	71 (28.1)	

Abbreviation: LNM, lymph nodes metastasis.

the basis of the median value. As shown in [Table 2](#), low CDH16 expression was significantly related to histological type ($P < 0.001$), signified large tumor size ($P = 0.036$), high tumor stage ($P = 0.001$), aggressive LNM ($P = 0.001$), and

Table 3 Univariate Logistic Regression Analysis for the Lymph Node Metastatic Risk

Clinicopathologic Features	OR	95% CI	P-Value
Age	0.614	0.424–0.891	0.010
Gender	1.541	1.016–2.337	0.042
Histological type	0.377	0.239–0.594	<0.001
Clinical stage	3.429	2.270–5.180	<0.001
Multifocality	1.381	0.950–2.008	0.091
CDH16 expression	0.521	0.359–0.757	0.001

Table 4 Multivariate Logistic Regression Analysis for the Lymph Node Metastatic Risk

Clinicopathologic Features	OR	95% CI	P-Value
Age	0.042	0.015–0.118	<0.001
Gender	1.344	0.811–2.226	0.251
Histological type	0.331	0.196–0.558	<0.001
Clinical stage	47.93	16.43–139.8	<0.001
CDH16 expression	0.574	0.369–0.893	0.014

advanced clinical stage ($P = 0.029$) (Table 2). Compared with the high expression group, the low CDH16 expression group was inclined to the classical subtype ($P < 0.001$, Table 2). However, no significant difference was found among CDH16 expression and age, gender, and multifocality ($P > 0.05$).

Low CDH16 Expression Could Enhance the Risk of LNM in PTC

The association between CDH16 expression and LNM in PTC was further investigated through logistic regression analyses. Univariate logistic regression analysis revealed that age ($OR = 0.614$, 95% $CI = 0.424–0.891$, $P = 0.01$), gender ($OR = 1.541$, 95% $CI = 1.016–2.337$, $P = 0.042$), histological type ($OR = 0.377$, 95% $CI = 0.239–0.549$, $P < 0.001$), clinical stage ($OR = 3.429$, 95% $CI = 2.270–5.180$, $P < 0.001$) and CDH16 expression ($OR = 0.521$, 95% $CI = 0.359–0.757$, $P = 0.001$) were significant variables for LNM in PTC (Table 3). Multivariate logistic analysis using these five parameters also indicated that age ($OR = 0.042$, 95% $CI = 0.015–0.118$, $P < 0.001$), histological type ($OR = 0.331$, 95% $CI = 0.196–0.558$, $P < 0.001$) and CDH16

Table 5 The Top 10 Most Significant Items of Gene Ontology (GO) Terms of the Co-Expression Genes of CDH16 in PTC

Category	Term	Count	P-Value
Biological processes			
GOTERM_BP_ALL	GO:0032501~multicellular organismal process	131	1.33E-08
GOTERM_BP_ALL	GO:0048731~system development	84	3.33E-08
GOTERM_BP_ALL	GO:0048856~anatomical structure development	88	6.62E-08
GOTERM_BP_ALL	GO:0007275~multicellular organismal development	95	1.58E-07
GOTERM_BP_ALL	GO:0032502~developmental process	100	4.98E-07
GOTERM_BP_ALL	GO:0048513~organ development	62	6.42E-06
GOTERM_BP_ALL	GO:0008015~blood circulation	15	2.24E-05
GOTERM_BP_ALL	GO:0003013~circulatory system process	15	2.24E-05
GOTERM_BP_ALL	GO:0021700~developmental maturation	11	3.35E-05
Cellular components			
GOTERM_CC_ALL	GO:0044459~plasma membrane part	69	5.53E-06
GOTERM_CC_ALL	GO:0005887~integral to plasma membrane	41	1.11E-04
GOTERM_CC_ALL	GO:0000267~cell fraction	38	1.53E-04
GOTERM_CC_ALL	GO:0031226~intrinsic to plasma membrane	41	1.78E-04
GOTERM_CC_ALL	GO:0005576~extracellular region	59	2.11E-04
GOTERM_CC_ALL	GO:0044421~extracellular region part	34	3.19E-04
GOTERM_CC_ALL	GO:0005624~membrane fraction	30	3.81E-04
GOTERM_CC_ALL	GO:0043005~neuron projection	17	5.42E-04
GOTERM_CC_ALL	GO:0044456~synapse part	14	5.82E-04
GOTERM_CC_ALL	GO:0005626~insoluble fraction	30	6.84E-04
Molecular functional			
GOTERM_MF_ALL	GO:0015267~channel activity	21	1.58E-04
GOTERM_MF_ALL	GO:0022803~passive transmembrane transporter activity	21	1.64E-04
GOTERM_MF_ALL	GO:0004252~serine-type endopeptidase activity	12	2.05E-04
GOTERM_MF_ALL	GO:0005215~transporter activity	41	3.23E-04
GOTERM_MF_ALL	GO:0016903~oxidoreductase activity, acting on the aldehyde or oxo group of donors	6	3.44E-04
GOTERM_MF_ALL	GO:0022836~gated channel activity	17	3.66E-04
GOTERM_MF_ALL	GO:0016491~oxidoreductase activity	28	4.05E-04
GOTERM_MF_ALL	GO:0005216~ion channel activity	19	5.44E-04
GOTERM_MF_ALL	GO:0008236~serine-type peptidase activity	12	7.03E-04
GOTERM_MF_ALL	GO:0017171~serine hydrolase activity	12	7.71E-04

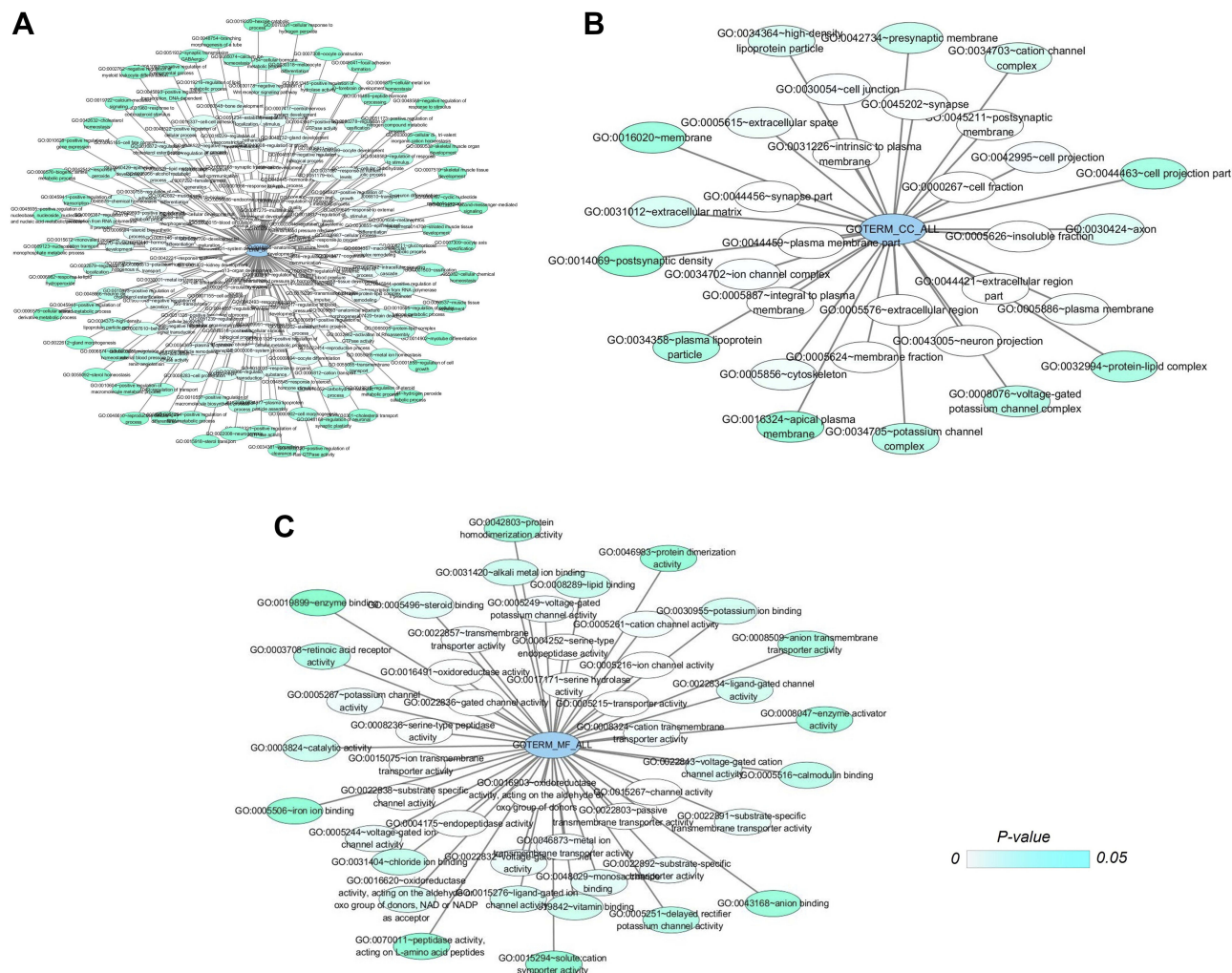


Figure 2 Networks of gene ontology (GO) terms of CDH16 co-expressed genes in PTC. **Notes:** (A) Network of biological processes (BPs) of CDH16 co-expressed genes. (B) Network of cellular components (CCs) of CDH16 co-expressed genes. (C) Network of molecular functions (MFs) of CDH16 co-expressed genes. In those networks, only terms with statistically significant ($P < 0.05$) are displayed. Each node represents a GO term, and the node color indicates the P-value of a GO term.

expression (OR = 0.574, 95% CI = 0.369–0.893, $P = 0.014$) were protective factors for LNM in PTC, whereas clinical stage (OR = 47.93, 95% CI = 16.43–139.8, $P < 0.001$) was an independent high-risk factor for LNM (Table 4). In summary, the downregulation of CDH16 expression can aggravate the risk of LNM in patients with PTC.

CDH16 Co-Expressed Genes and Bioinformatics Analyses

Based on MEM, 5000 of the most significant CDH16 co-expressed mRNA genes were selected. The data from the cBioPortal for Cancer Genomics showed 20,033 CDH16 co-expressed mRNA genes in the PTC patients. A total of 1495

Table 6 KEGG Pathways Enriched by Co-Expression Genes of CDH16 in PTC

Category	Term	Count	P-Value
KEGG_PATHWAY	hsa00380:Tryptophan metabolism	5	0.014
KEGG_PATHWAY	hsa00350:Tyrosine metabolism	5	0.020
KEGG_PATHWAY	hsa05200:Pathways in cancer	14	0.047
KEGG_PATHWAY	hsa04912:GnRH signaling pathway	6	0.083
KEGG_PATHWAY	hsa00280:Valine, leucine and isoleucine degradation	4	0.086
KEGG_PATHWAY	hsa00140:Steroid hormone biosynthesis	4	0.095

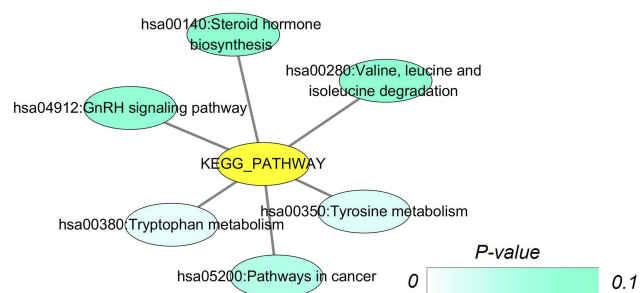


Figure 3 Network mappings of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways of CDH16 co-expressed genes in PTC.

Notes: In this network, all terms related are displayed. Each node represents a KEGG term, and the node color indicates the P-value of a KEGG term.

genes were obtained by excluding the genes with an absolute value of the Pearson's correlation coefficient <0.4 . The intersection between the results of the two databases was collected,

and 340 reliable co-expressed genes were extracted for further study. GO and KEGG analyses were performed to determine the biological processes involved in PTC tumorigenesis. The results of the GO term analysis (Table 5) revealed that the genes are involved in the following: (1) biological processes (Figure 2A), including the multicellular organismal process, the system development, the anatomical structure development, the developmental process, the organ development, etc.; (2) cellular components (Figure 2B), such as plasma membrane part, integral to plasma membrane, cell fraction, and intrinsic to plasma membrane; and (3) molecular functions (Figure 2C), such as channel activity, passive transmembrane transporter activity, serine-type endopeptidase activity, and transporter activity. The results of the KEGG pathway analysis showed that CDH16 participated in the functional regulation of six signaling pathways (Table 6). The relatively important

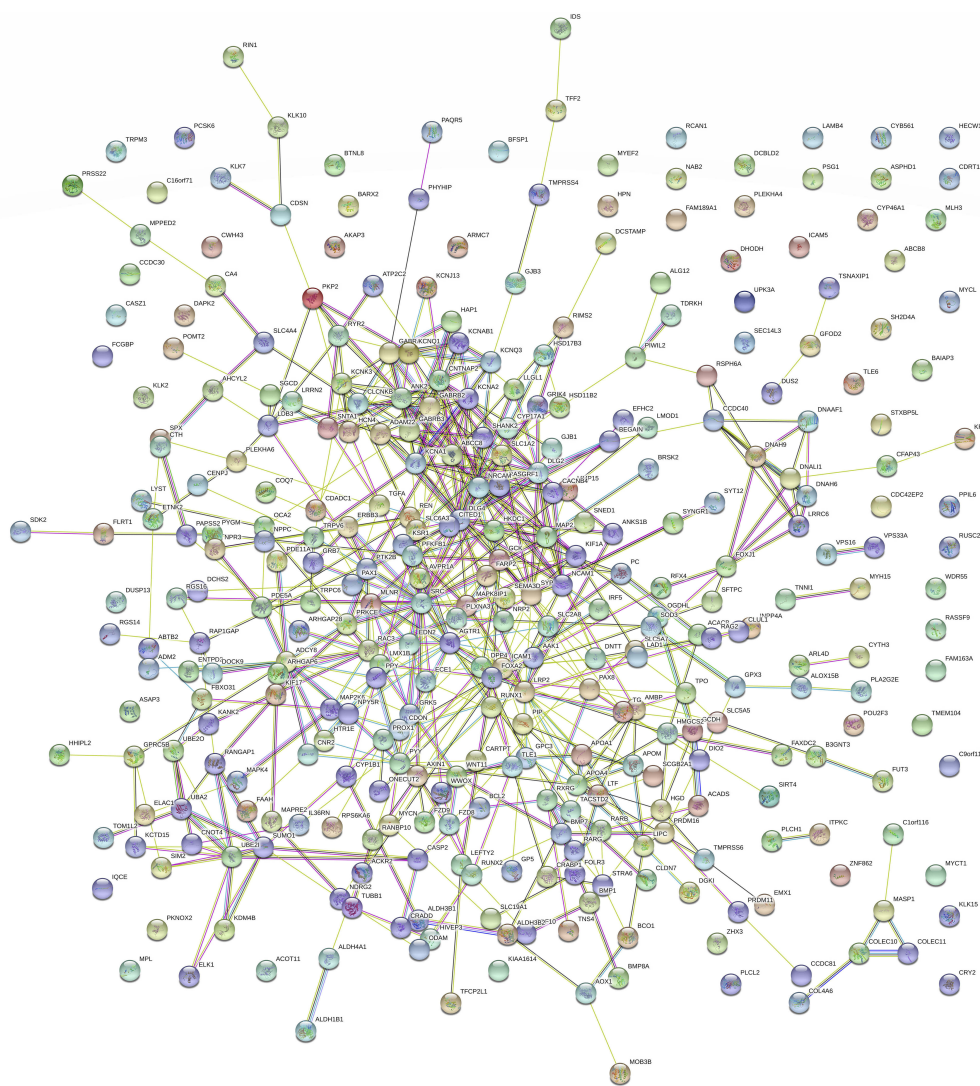


Figure 4 Network of protein-protein interaction (PPI) analyses of CDH16 co-expressed genes in PTC.

Table 7 The Top 10 with Combined Co-Expression Score That the Protein–protein Interaction (PPI) of CDH16 Co-Expression Genes in PTC Enriched in Significant Signaling Pathways

Node1	Node2	Node1 STRING Internal ID	Co-Expression	Experimentally Determined Interaction	Automated Text Mining	Combined Score
CRADD	CASP2	4449892	0	0.993	0.890	0.999
RANGAPI	UBE2I	4448176	0.075	0.996	0.878	0.999
UBE2I	UBA2	4441792	0.091	0.992	0.945	0.999
SUMO1	UBE2I	4446000	0.194	0.98	0.968	0.999
VPS16	VPS33A	4445392	0.747	0.996	0.933	0.999
SUMO1	UBA2	4446000	0.142	0.915	0.912	0.999
RANGAPI	SUMO1	4448176	0.109	0.875	0.916	0.998
BCL2	CASP2	4446777	0	0.993	0.262	0.995
KCNA2	KCNAB1	4449656	0.118	0.814	0.689	0.994
KCNAB1	KCNA1	4449075	0.144	0.732	0.725	0.992

pathways include tryptophan metabolism, tyrosine metabolism, and pathways in cancer (Figure 3). PPI analyses were performed and the interaction nodes are shown in Figure 4. The top 10 nodes with the highest overall scores are listed in Table 7. We also focused on the genes enriched in “pathways in cancer”. Ten interaction nodes were obtained based on the results of PPI analyses (Figure 5). The findings suggested that WNT11, FZD9, FZD8, AXIN1, RXRG, all of which are common genes in cancer-related pathways, might play important connective roles in cancer initialization (Table 8) and therefore were further analyzed. According to the TCGA

database, the results of Pearson’s correlation analyses demonstrated that AXIN1, RXRG exhibited a potential negative correlation with CDH16 expression, whereas WNT11, FZD9, FZD8 exhibited a possible positive correlation with CDH16 (Figure 6)

Discussion

Thyroid cancer, which is one of the most common endocrine malignancies, has become the fastest growing type of cancer worldwide.¹ This disease can be classified into several different subtypes according to molecular etiology.^{26,27} Patients with PTC have a statistically better prognosis and longer survival than those with other subtypes.⁴ However, the high incidence of LNM in PTC patients can significantly increase the risk of locoregional recurrence and mortality.^{7,8} Searching for potential biomarkers can provide new clinical insights into predicting the metastasis status of patients with PTC.

CDH16 plays an indispensable role in human development and growth.^{18,20} Thedieck et al found that CDH16 can facilitate tubulogenesis during human renal development and can be detected in the distal tubules of adults’ kidneys.²³ Moreover, Morizane et al further proved that CDH16-positive cells derived from embryonic stem cells can reproduce tubular structures and differentiate into renal tubular cells.²⁸ In addition, the CDH16 expression is associated with the fully differentiated state of the thyroid cells²⁹ and partially influences the thyroid follicular polarity.³⁰ However, the abnormal CDH16 expression can lead to tumorigenesis. Thedieck et al reported CDH16 downregulation in renal cell carcinoma and suggested the role of CDH16-related cell adhesion molecule in tumor suppression.²³ As for the thyroid cancer, Cali et al confirmed that the downregulated CDH16

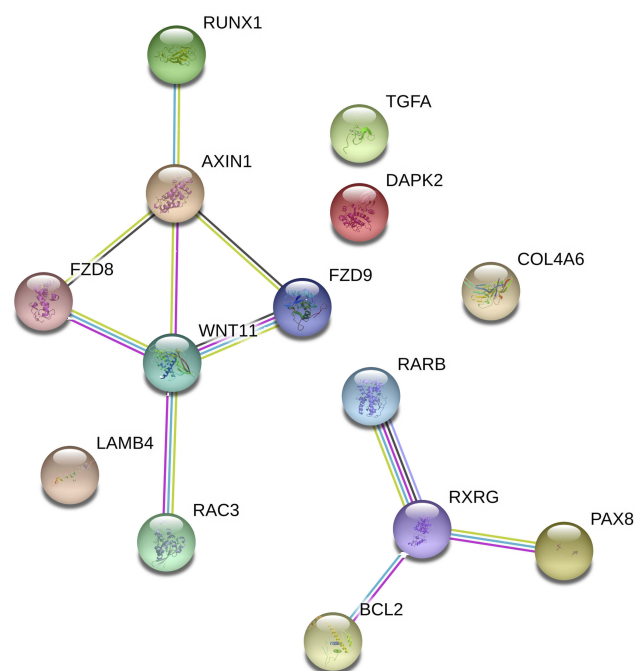
**Figure 5** PPI network analyses of genes coexpressed in CDH16 in pathways in cancer.

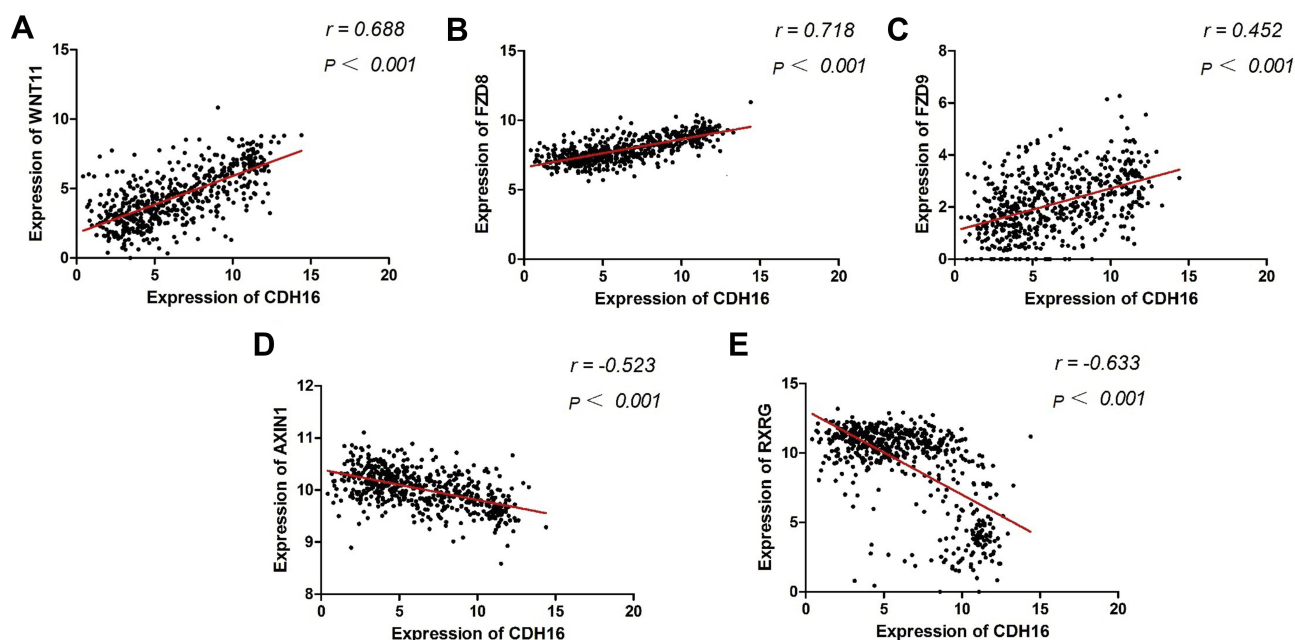
Table 8 The Top 10 with Combined Co-Expression Score That the Protein-protein Interaction (PPI) of CDH16 Co-Expression Genes in PTC Enriched in Pathways in Cancer

Node1	Node2	Node1 STRING Internal ID	Co-Expression	Experimentally Determined Interaction	Automated Textmining	Combined Score
RXRG	RARB	4442297	0.063	0.744	0.674	0.977
FZD8	WNT11	4444438	0	0.266	0.643	0.971
FZD9	WNT11	4441555	0.062	0.262	0.627	0.97
WNT11	RAC3	4439769	0	0.063	0.21	0.919
BCL2	RXRG	4446777	0	0.064	0	0.902
RUNX1	AXIN1	4437664	0	0	0.064	0.902
RXRG	PAX8	4442297	0	0.05	0.226	0.84
WNT11	AXIN1	4439769	0	0.081	0.678	0.692
FZD8	AXIN1	4444438	0.062	0	0.576	0.586
FZD9	AXIN1	4441555	0.063	0	0.519	0.53

expression can be used as a marker to monitor thyroid cancer.²⁵ In the present study, CDH16 expression was significantly downregulated in PTC. This finding is consistent with previous studies on human cancers indicating that CDH16 serves as a tumor suppressor in different kinds of human cancer.^{23,25} To the best of our knowledge, our study is the first to report the significant role of CDH16 in PTC.

We first reported that CDH16 plays an important role in LNM in PTC. qRT-PCR detection and TCGA RNA-Seq data were used to confirm that the CDH16 expression was

significantly downregulated in PTC tumor tissues compared with that in noncancerous thyroid tissues. In addition, this downregulation of CDH16 was associated with unfavorable clinicopathological factors, such as aggressive LNM and high clinical stage. Subsequent logistic regression analyses showed that the downregulated expression of CDH16 can be an independent high-risk factor for LNM in PTC patients. The results confirmed that CDH16 acted as a tumor suppressor gene in PTC and was involved in the progression of LNM.

**Figure 6** Correlations between the hub genes and the expression of CDH16 in PTC.

Notes: (A) Correlation between expression of WNT11 and expression of CDH16 in PTC. $r = 0.688$, $P < 0.001$. (B) Correlation between expression of FZD8 and expression of CDH16 in PTC. $r = 0.718$, $P < 0.001$. (C) Correlation between expression of FZD9 and expression of CDH16 in PTC. $r = 0.452$, $P < 0.001$. (D) Correlation between expression of AXIN1 and expression of CDH16 in PTC. $r = -0.523$, $P < 0.001$. (E) Correlation between expression of RXRG and expression of CDH16 in PTC. $r = -0.633$, $P < 0.001$.

The molecular mechanisms of CDH16 in PTC were also explored in this study. We used GO and KEGG analyses to compare the enrichment of CDH16 co-expressed genes in PTC patients. The results suggested that the co-expressed genes play important roles in biological processes, cellular components, and molecular functions. According to the result of the KEGG pathways analysis, the genes were more concentrated on the pathways in cancer. The expression of CDH16 was positively related to WNT11, FZD9, FZD8 and negatively related to AXIN1 and RXRG. In conclusion, CDH16 performs regulatory functions in PTC through complex protein–protein interaction with the aforementioned genes.

Despite the substantial findings, this research still exhibits some limitations. First, the findings are mainly theoretical data that should be validated through in vitro and in vivo experiments. Second, the regulatory mechanism between CDH16 and its co-expressed genes should be further investigated.

In conclusion, CDH16 expression was downregulated in PTC tissues. The low expression of CDH16 can aggravate the risk of LNM in PTC. Moreover, CDH16 can collaborate with co-expressed genes, such as RXRG, WNT11 and so on, to regulate the pathways in cancer to change the LNM status. This research provides reliable molecular theories for further studies on the molecular mechanisms of CDH16 in PTC.

Ethical Approval

Ethical approval for this study was obtained from the Ethical Committee of the Second Affiliated Hospital of Wenzhou Medical University.

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Disclosure

The authors report no conflicts of interest in this work.

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