

Analysis of the expression and potential molecular mechanism of interleukin-1 receptor antagonist (IL1RN) in papillary thyroid cancer via bioinformatics methods

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Abstract

Background: Interleukin-1 receptor antagonist (IL1RN) has been reported as a biomarker of many cancers. However, the biological function of IL1RN in papillary thyroid carcinoma (PTC) remains undetermined.

Methods: We obtained IL1RN expression data from The Cancer Genome Atlas (TCGA) database. Enrichment analysis of coexpressed genes and IL1RN methylation analysis were performed via LinkedOmics. The correlations between IL1RN and immune infiltrates were investigated via ESTIMATE, TIMER and TISIDB. We analyzed the association of IL1RN expression with pancancer overall survival (OS) via Gene Expression Profiling Interactive Analysis (GEPIA).

Results: IL1RN showed higher expression levels and lower methylation levels in PTC tissues than in normal tissues. Higher IL1RN expression was significantly associated with shorter progression-free survival (PFS), advanced tumor stage, tumor metastasis, increased incidence of BRAF mutations, and decreased incidence of N-RAS and H-RAS mutations. Genes coexpressed with IL1RN participate primarily in immune-related pathways. IL1RN expression was positively correlated with immune infiltration, tumor progression and poor overall survival for all cancers.

Conclusions: IL1RN is a good prognostic and diagnostic biomarker for PTC. IL1RN may promote thyroid cancer progression through immune-related pathways. Methylation may act as an upstream regulator of IL1RN expression and biological function. Additionally, IL1RN was shown to have broad prognostic value in a pancancer cohort.

1 Introduction

Thyroid carcinoma (THCA) is the most common endocrine malignancy worldwide [1]. The incidence of THCA has increased sharply over the past 3 decades [2]. Papillary thyroid carcinoma (PTC) is the major subtype of THCA, accounting for more than 90% of cases [3]. The clinical course of PTC is generally indolent, and the cancer-specific mortality of PTC is low compared to that of other cancers [4]. However, the incidence of cervical lymph node metastasis is high, which leads to local recurrence and poor prognosis [5]. The main task for the future is to identify high-risk patients and to give them appropriate treatment and care [6]. Therefore, further studies are required to explore the underlying mechanisms of tumorigenesis as well as identify additional biomarkers that predict prognosis and serve as therapeutic targets.

The interleukin-1 (IL-1) family of cytokines are the most effective molecules in the innate immune system [7]. IL-1 receptor antagonist (IL1RN) was initially found as a natural antagonist of IL-1 [8]. IL1RN is structurally related to IL-1 α and IL-1 β but binds to IL-1 receptors on various target cells without inducing any discernible biological responses [9]. The balance between IL1RN and IL-1 plays a crucial role in many diseases, including cancer [10]. IL1RN has been studied in several cancers, including prostate carcinoma

[11–12], cervical carcinoma [13], gastric carcinoma [14], bronchogenic carcinoma [15], endometrial cancer [16], lung cancer [17], ovarian cancer [18], oral malignancies [19], leukemia [20], and other cancers.

Two structural variants of IL1RN have been described: the soluble extracellular form (sIL-1ra) and the intracellular form (icIL-1ra) [4]. Niedzwiecki, S. and colleagues assayed the serum levels of IL1RN in thyroid cancer patients. They measured preoperative IL1RN serum levels of patients with thyroid cancer, and the results showed that the serum concentrations of sIL-1ra were significantly higher in anaplastic carcinoma (ATC) and follicular carcinoma (FTC) patients [21]. To the best of our knowledge, no reports have been published to date concerning IL1RN expression in thyroid tissue. The objective of this study was to investigate the expression of IL1RN in normal and papillary thyroid cancer tissues by performing bioinformatics analysis to elucidate its possible role in tumor progression.

2 Materials And Methods

2.1 Expression level analysis

The cohort that comprised 512 PTC and 58 normal thyroid samples was obtained from The Cancer Genome Atlas (TCGA) (<https://tcga-data.nci.nih.gov/tcga/>). The clinical data, normalized RNA expression data, DNA methylation data and simple nucleotide variation data were downloaded from the TCGA data portal.

The gene expression profiles of four independent datasets (GSE3467, GSE33630, GSE58545, and GSE60542) were downloaded from the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) database (<http://www.ncbi.nlm.nih.gov/geo/>).

The immunohistochemical staining images of IL1RN in PTC and normal tissues were obtained from The Human Protein Atlas (<https://www.proteinatlas.org>).

2.2 Diagnostic and prognostic value analysis

ROC curves were plotted, and the area under the ROC curve was calculated using the ROCR package in R. The patients were divided into a high IL1RN expression group (H-IL1RN) and a low IL1RN expression group (L-IL1RN) according to the best matched value for the survival analysis. Kaplan-Meier survival curves were generated by the R survival package. The primary end point of the study was progression-free survival (PFS). Univariate and multivariate analyses were performed using the Cox regression model to assess the significance of various variables to survival. A chi-square test was performed to compare the clinical characteristics between the H-IL1RN group and the L-IL1RN group.

2.3 Gene functional enrichment analysis

We identified the genes that were significantly positively or negatively correlated with IL1RN using the LinkedOmics website (<http://www.linkes.org/>).

The top 50 positively correlated genes and the top 50 negatively correlated genes were selected to build the heatmaps.

These genes were inputted into the GO and KEGG websites to obtain the enriched GO terms and significant KEGG pathways. GO function annotation analysis was performed based on the GO database (<http://geneontology.org/page/go-database>), and KEGG pathway annotation analysis was performed based on the KEGG database (<http://www.kegg.jp/kegg/ko.html>).

The protein-protein interaction (PPI) network with a confidence > 0.7 was constructed using STRING (<https://string-db.org>) and CytoScape version 3.7.2.

2.4 Tumor immunology analysis

ESTIMATE used the single-sample gene-set enrichment analysis (ssGSEA) score to quantify the enrichment levels of immune gene signatures in tumors. The stromal, immune and ESTIMATE scores of TCGA PTC samples were computed using ESTIMATE, and then the tumor purity of each sample was inferred.

The Tumor Immune Estimation Resource (TIMER) web server (<https://cistrome.shinyapps.io/timer/>), a comprehensive analytic web tool, was used to analyze the correlation of IL1RN with infiltration of immune cells, including B cells, CD8 + T cells, CD4 + T cells, macrophages, neutrophils and dendritic cells (DCs).

We used TISIDB (<http://cis.hku.hk/TISIDB/>), a web portal for investigation of tumor and immune system interaction, to determine the Spearman correlation between IL1RN expression and 28 TIL types, chemokines, immune-activating cytokines, immunosuppressive cytokines, major histocompatibility complex (MHC) molecules, and chemokine receptors.

2.5 Methylation-related analysis

DNA methylation data were downloaded from the data portal of TCGA (<https://portal.gdc.cancer.gov/>), and the DNA methylation levels of the PTC and control groups were then compared. Spearman correlation analysis was conducted to examine the associations between the methylation density and gene expression and between the methylation density and tumor purity. Additionally, we analyzed the correlation between IL1RN expression and the methylation level of each CpG site using the Spearman correlation test. The analysis of the relationship between methylation and the clinical characteristics and the GO and KEGG analyses were performed using linkedOmics.

2.6 Pancancer analysis

The pancancer expression and survival analysis of IL1RN was performed using the online software Gene Expression Profiling Interactive Analysis (GEPIA) (<http://gepia.cancer-pku.cn/>). We used TISIDB to analyze the relationships between IL1RN expression and overall survival (OS) in pancancer. Kaplan-Meier (K-M) curves were generated for pancancer using the TCGA cohort data.

2.7 Related mutant gene analysis

The analysis of the relationship between IL1RN expression and PTC mutations was performed by linkedOmics. The IL1RN expression level was further compared between the groups with wild-type and mutant versions of BRAF, NRAS and HRAS.

2.8 Statistical analysis

The *t*-test and Mann-Whitney U test were used for comparisons between two groups. The chi-square test was used to assess the differences in clinical parameters between the L-IL1RN and H-IL1RN groups. The Pearson and Spearman methods were used for correlation analysis. The log-rank method was used to calculate the significant *p*-values related to survival. R software (v3.6.0) and SPSS version 25.0 software were used for statistical processing. Visualization of data was performed with GraphPad Prism V.8.0 and R software. $p < 0.05$ was considered significant.

3 Results

3.1 The differential expression and diagnostic value of IL1RN

The expression levels of IL1RN were initially analyzed in the TCGA cohort consisting of 512 PTC samples and 58 normal samples (Figure 1A). Furthermore, expression data from four independent PTC cohorts obtained from the Gene Expression Omnibus (GEO) (GSE3467, GSE33630, GSE58545, and GSE60542) were employed for validation (Figure 5A-D). Both the GEO and TCGA patient cohorts had significantly higher IL1RN mRNA expression in PTC tissues than in normal tissues ($P < 0.05$). Elevated levels of IL1RN protein expression were observed in PTC tissues compared with those in normal tissues by immunohistochemistry performed using the IL1RN antibody CAB9633. (Figure 1C)

ROC curves were constructed to evaluate the diagnostic value of IL1RN for PTC. The area under the ROC curve of the TCGA cohort was 0.7084 (Figure 1B) and that of each GEO cohort was 0.9753, 0.7279, 0.8868, and 0.8384, respectively. (Figure 5E-H) These results suggest the good positive diagnostic value of IL1RN for PTC.

3.2 Prognostic value and clinical significance of IL1RN

The PTC samples from the TCGA cohort were divided into two groups according to the IL1RN expression level: the high-expression group (H-IL1RN) ($n = 116$) and the low-expression group (L-IL1RN) ($n = 385$). Differences in progression-free survival (PFS) between the two groups were analyzed by Kaplan-Meier analysis. (Figure 1D) The results showed that patients with high IL1RN expression had significantly shorter PFS than those with low expression ($P = 0.0034$). However, the data for the multivariate analysis by the Cox regression model suggested that IL1RN expression was not a significant independent prognostic risk indicator for PTC (hazard ratio, 1.033; 95% confidence interval, 0.999–1.068, $p > 0.05$) (Table 1). Age was an independent predictor of poor prognosis ($P \leq 0.05$).

To examine the clinical significance of IL1RN in PTC, the relationship between the expression level of IL1RN and clinical characteristics was investigated by the chi-square test (Table 2). The results suggested that IL1RN expression was significantly associated with age, clinical stage, N stage, T stage, pathologic type, BRAF mutations and RAS mutations.

3.3 Gene coexpression and pathway enrichment analysis

We identified the genes that are significantly positively or negatively related to IL1RN. The result is presented as a volcano plot (Figure 2A). Red and green colors indicate a positive and negative correlation, respectively. The top 50 positively (Figure 2B) or negatively (Figure 2C) correlated genes are depicted by heatmaps. In addition, 2 hub genes (CCL20 and FN1) were identified from the protein–protein interaction (PPI) network (Figure 2D).

GO enrichment analysis revealed that the identified genes were mainly enriched for the GO biological process terms “neutrophil mediated immunity”, “positive regulation of cytokine production”, “adaptive immune response” and “immune response-regulating signalling pathway” (Figure 2E). Further KEGG enrichment analysis suggested that the identified genes were mainly involved in pathways associated with “cell adhesion molecules (CAMs)”, “cytokine-cytokine receptor interaction”, and “phagosome” (Figure 2F).

3.4 Immune-related analysis of IL1RN

Using ESTIMATE, the association between IL1RN expression and immune infiltrates was analyzed. We found that the H-IL1RN group showed a higher immune score (Figure 3B) and ESTIMATE score (Figure 3C) and lower tumor purity (Figure 3D) than the L-IL1RN group. No significant difference in the stromal score was found between the two groups (Figure 3A). The correlations between the IL1RN expression level and the infiltration of six immune cell types in PTC were estimated. The results demonstrated that the expression of IL1RN was found to be significantly correlated with the infiltration of immune cells, especially neutrophils and dendritic cells (Figure 3E). Correlations between IL1RN expression and 28 TIL types (Figure 3F), chemokines (Figure 3G), immune-activating cytokines (Figure S2A), immunosuppressive cytokines (Figure S2B), MHC molecules (Figure S2C), and chemokine receptors (S2D) in pancancer are shown in heatmaps. The correlation analysis results showed a good correlation between IL1RN expression and immune-related molecules.

3.5 Methylation-related analysis

The data showed that the degree of IL1RN DNA methylation was higher in carcinoma tissues than in normal tissues ($P < 0.0001$) (Figure 4A). To further determine the role of methylation, we performed a correlation analysis between the DNA methylation levels of IL1RN and the expression levels of IL1RN. As expected, DNA methylation was negatively correlated with IL1RN expression ($R = -0.600$, $P = 2.29 \times 10^{-50}$) (Figure 4B). Tumor purity was significantly positively associated with IL1RN methylation ($R = 0.447$, $P = 7.49 \times 10^{-32}$) (Figure 4C). Patients with an advanced clinical stage (Figure 4D), advanced T stage (Figure

4E) and lymph node metastasis (Figure 4F) tended to have decreased IL1RN methylation levels. In the pathological subtype of PTC, the tall cell type variant of PTC had the lowest degree of methylation, followed by the classical type, and the follicular variant of PTC had the highest degree of methylation (Figure 4G). To further explore the associated functional changes in IL1RN methylation, GO (Figure 4I) and KEGG (Figure 4F) enrichment analyses were performed. The results showed that methylated IL1RN had the opposite effect of IL1RN. Furthermore, the most significant methylation sites are shown in Table 3.

3.6 Pancancer analysis of IL1RN

We analyzed the IL1RN expression levels in different kinds of tumors via the GEPIA platform (Figure 6A). The results showed that the expression levels of IL1RN varied greatly in different cancer types. Of the 33 cancer types tested, 12 cancer types were associated with significantly increased IL1RN expression. Subsequently, the relationship between IL1RN and OS in pancancer was investigated (Figure 6B). Kaplan-Meier survival curves for the high IL1RN expression group (4745 patients) and low IL1RN expression group (4750 patients) indicated that increased IL1RN expression was associated with a shorter survival time in pancancer (HR=1.6, $P<0.0001$) (Figure 6C). The results showed that increased IL1RN expression levels were significantly associated with shorter OS in kidney renal clear cell carcinoma (KIRC) (Figure 6D), brain lower grade glioma (LGG) (Figure 6E), pancreatic adenocarcinoma (PADD) (Figure 6F) and uterine corpus endometrial carcinoma (UCEC) (Figure 6G).

3.7 Related mutant gene analysis

The analysis of the relationship between IL1RN expression and PTC mutations was performed by linkedOmics (Figure S1A). The IL1RN expression level was further compared between the groups with wild-type and mutant variants of BRAF, NRAS and HRAS (Figure S1B-D). The expression of IL1RN was significantly higher in PTC with the BRAF mutant than in PTC with the wild type (Figure S1B). However, mutated NRAS (Figure S1C) and mutated HRAS (Figure S1D) were correlated with decreased IL1RN expression.

4 Discussion

In this study, we first revealed high IL1RN expression in PTC tissues and its diagnostic and prognostic value by integrated bioinformatics analysis. To further explore the potential function of IL1RN, we identified genes related to IL1RN expression, and functional enrichment analyses were conducted. The results of the KEGG pathway and GO analyses revealed the significant enrichment of genes in immune-related pathways. Our work further demonstrated that high IL1RN expression was significantly associated with decreased tumor purity and increased immune infiltration. Through methylation-related analysis, we determined that DNA methylation might be a regulatory mechanism of IL1RN. In addition, the analysis of the relationship between IL1RN expression and PTC mutations showed that high IL1RN expression was associated with mutated BRAF, wild-type NRAS and wild-type HRAS. Finally, IL1RN shows superior prognostic value for various cancers according to the pancancer analysis.

Niedzwiecki et al [21] revealed that the serum levels of IL1RN were upregulated in patients with ATC and PTC, but no statistically significant difference was found in PTC. We found that the expression of IL1RN was increased in PTC tissues compared with that in normal tissues in 1 TCGA cohort and 4 GEO cohorts and suggested that IL1RN might be used as a potential diagnostic biomarker in PTC. Our results were consistent with those of previous reports on the upregulation of IL1RN in cervical carcinoma [13], gastric cancer [14], lung cancer [15], and endometrial cancer tissues [16]. These findings are also basically consistent with the results of our pancancer study. In contrast to these findings, IL1RN exhibited low expression in oral squamous cell carcinomas (OSCCs) [19].

The results of this study showed the value of IL1RN as a clinical biomarker in PTC and emphasized its potential as a prognostic biomarker in PTC patients. PTC patients with high IL1RN expression had decreased PFS compared to those with low IL1RN expression. Furthermore, high expression of IL1RN was significantly correlated with clinical stage, lymph node metastasis and pathological type. Various studies have highlighted the significant association between IL1RN expression and poor cancer prognosis; however, the results of these studies are conflicting. Van Le et al. [16] revealed that there was a significant correlation between the stage of endometrioid carcinoma and the expression of IL1RN. In a study of colorectal cancer, serum IL1RN levels were correlated with tumor size, local and distant metastasis and vascular infiltration [32]. An increase in IL1RN expression was correlated significantly with lymph node and liver metastases in gastric carcinoma [14]. In contrast, low levels of IL1RN have been associated with increased disease severity in myeloma [22], colorectal cancer [23] and prostate cancer [24].

The underlying mechanism of IL1RN in cancer development and progression is complicated and unclear. In this study, functional enrichment analysis of genes coexpressed with IL1RN showed that IL1RN participates in immune-related biological processes. Consistent with our findings, previous studies have focused on the role of IL1RN in tumor immunity. A study of gastric carcinoma [14] pointed out that on the one hand, IL1RN may promote tumor growth via the impairment of cellular immunity; on the other hand, IL1RN enables malignant cells to escape host immune responses. Smith, D. R. et al. [15] revealed that increased IL1RN in bronchogenic carcinoma is not accompanied by increased IL-1 β activity. The altered balance of IL1RN and IL-1 β may result in impaired immune surveillance and cytotoxic activity. An experimental study of human glioblastoma cells showed that IL1RN secreted by tumor cells can counteract IL-1 function, which represents a potential escape mechanism that supports cancer growth [31]. In our study, IL1RN was significantly positively correlated with lymph node metastasis and tumor stage, so we speculated that IL1RN might also promote tumor aggressiveness and poor prognosis through immune-related mechanisms in PTC.

IL1RN is an endogenous natural antagonist of IL-1[8], so the discovery of the interaction between IL1RN and IL-1 family molecules is a breakthrough in exploring the function of IL1RN. ONOZAKI et al. [25] reported that IL-1 is a cytotoxic factor against several tumor cell lines. IL-1 may enhance cytotoxic T cell activity [29], the tumoricidal capacities of natural killer (NK) cells [30], and monocyte-mediated cytotoxicity [37]. Because IL-1 is critical for tumor immunity, elevation of IL1RN expression may result in a

general environment favourable to tumor cells and enhance the metastatic and recurrence potential of tumors by changing local IL-1-dependent pathways.

IL-1 β has been reported as an anticancer factor that acts to suppress proliferation and reduce the invasive potential of human PTC cells [26]. sIL-1ra has been shown to block IL-1 function by binding to IL-1 receptors at the cell membrane level [27]. IclIL-1ra is postulated to inhibit intracellular IL-1 activity [28]. Therefore, whether IL1RN can block the function of IL-1 and inhibit the anticancer effect of IL-1 β in PTC is worth further study.

In our study, IL1RN showed a significant positive correlation with immune cells, which may be because a variety of immune cells can produce IL1RN through the stimulation of cytokines. Neutrophils produce IL1RN in response to granulocyte/macrophage colony stimulating factor (GM-CSF) and tumor necrosis factor- α (TNF- α) [34]. IL-4 and IL-10 have been reported to increase the production of IL1RN by human monocytes [35]. Yanagawa, H.et, al. reported that IL-13 increases IL1RN production by human alveolar macrophages [36]. It was reported that the apoptotic cell death of monocytes was enhanced by administration of recombinant IL1RN [33]. Our study also showed a significant correlation between IL1RN expression and various cytokines, which implied that IL1RN may play a complex role in tumor immunity.

Our study suggests that methylation is a pretranscriptional regulatory mechanism for IL1RN. The methylation level of IL1RN was negatively correlated with its expression level and was also correlated with the disease stage, lymph node metastasis, and pathological type.

IL1RN is likely to be a potential biomarker associated with the diagnosis and prognosis of PTC. However, although the diagnostic value, prognostic value and molecular functions of IL1RN in PTC have been analyzed through bioinformatics methods, the conclusions have not yet been confirmed by experiments. Therefore, further research is necessary to explore the role of IL1RN in PTC and the pharmacological value of IL1RN as a therapeutic target.

5 Conclusions

IL1RN is a good prognostic and diagnostic biomarker of PTC. IL1RN may promote thyroid cancer progression through immune-related pathways. Methylation may act as an upstream regulator of IL1RN expression and biological function. Additionally, IL1RN showed broad prognostic value in an analysis of a pancancer cohort.

Declarations

1 Ethics approval and consent to participate

Not applicable

2 Consent for publication

Not applicable

3 Availability of data and materials

The datasets analysed during the current study are available in The Cancer Genome Atlas (TCGA) (<https://tcga-data.nci.nih.gov/tcga/>) or the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) database (<http://www.ncbi.nlm.nih.gov/geo/>).

4 Competing interests

The authors declare that they have no competing interests.

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6 Authors' contributions

ZX designed the analytical strategies, performed data analyses and wrote the manuscript. XL performed data analysis and wrote the manuscript. YZH, SW, SYW, JS, YCH, YL and SJX performed data analysis. JZ conceived the research and wrote the manuscript.

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Abbreviations

IL1RN	interleukin-1 receptor antagonist
PTC	papillary thyroid carcinoma
TCGA	The Cancer Genome Atlas
OS	overall survival
GEPIA	Gene Expression Profiling Interactive Analysis
PFS	progression-free survival
THCA	thyroid carcinoma
IL-1	interleukin-1
sIL-1ra	the soluble extracellular form of IL1RN
icIL-1ra	the intracellular form of IL1RN
ATC	anaplastic carcinoma
FTC	follicular carcinoma
NCBI	the National Center for Biotechnology Information
GEO	Gene Expression Omnibus
H-IL1RN	high IL1RN expression group
L-IL1RN	low IL1RN expression group
PPI	protein-protein interaction
ssGSEA	single-sample gene-set enrichment analysis
TIMER	Tumor Immune Estimation Resource
DCs	dendritic cells
MHC	major histocompatibility complex
K-M	Kaplan-Meier
CAMs	cell adhesion molecules
KIRC	kidney renal clear cell carcinoma
LGG	lower grade glioma
PADD	pancreatic adenocarcinoma
UCEC	uterine corpus endometrial carcinoma
OSCCs	oral squamous cell carcinomas
NK	natural killer

GM-CSF	granulocyte/macrophage colony stimulating factor
TNF- α	tumor necrosis factor- α

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Tables

Table 1

Variables	Univariate analysis		Multivariate analysis	
	Hazard ratio (95%CI)	P value	Hazard ratio (95%CI)	P value
Age(y≥55/<55)	3.958(2.274–6.891)	<0.001	2.582(1.312–5.083)	0.006
Gender(male/female)	1.471(0.834–2.592)	0.182	1.098(0.604–1.997)	0.758
Clinical stage	1.736(1.380–2.185)	<0.001	1.301(0.968–1.748)	0.081
Radiation therapy	1.305(0.728–2.343)	0.372	1.205(0.651–2.233)	0.553
IL1RN	1.058(1.024–1.093)	<0.001	1.033(0.999–1.068)	0.059

Table 2

Clinical parameters	L-IL1RN(n=385,%)	H-IL1RN(n=116,%)	P-value
Age(y)			
<55	261(67.8)	73(62.9)	0.330
≥55	124(32.2)	43(37.1)	
Gender			
Female	282(73.2)	84(72.4)	0.859
Male	103(26.8)	32(27.6)	
Clinical stage			
I	224(58.5)	57(49.1)	0.001
II	47(12.3)	5(4.3)	
III	77(20.1)	34(29.3)	
IV	35(9.1)	20(17.2)	
NA	2	0	
Metastasis			
M0	208(97.2)	74(96.1)	0.635
M1	6(2.8)	3(3.9)	
NA	171	39	
N classification			
N0	189(54.8)	40(37.7)	0.002
N1	156(45.2)	66(62.3)	
NA	40	10	
T classification			
T1	120(31.2)	22(19.3)	<0.001
T2	135(35.1)	29(25.4)	
T3	116(30.1)	54(47.4)	
T4	14(3.6)	9(7.9)	
NA	0	2	
Pathologic type			
Classical	268(69.6)	87(75.0)	<0.001
Follicular	94(24.4)	7(6.0)	
Tall Cell	17(4.4)	19(16.4)	
Other	6(1.6)	3(2.6)	
BRAF			
Wild	179(48.5)	16(14.3)	<0.001
Mutation	190(51.5)	96(85.7)	
NA	16	4	
RAS			
Wild	311(84.3)	110(98.2)	<0.001
Mutation	58(15.7)	2(1.8)	
NA	16	4	

Table 3

Methylation site	Spearman r	P value
cg01467417	-0.597	7.76E-50
cg02543462	-0.406	2.53E-21
cg01991967	-0.19	1.81E-05
cg03989987	-0.164	2.28E-04
cg10938446	-0.116	0.009
cg03703171	-0.107	0.016
cg11783497	-0.1	0.016
cg17669033	0.027	0.548
cg23041410	-0.022	0.625
cg06658391	0.018	0.687
cg25928199	NA	NA
cg25265126	NA	NA
cg02377053	NA	NA

Figures

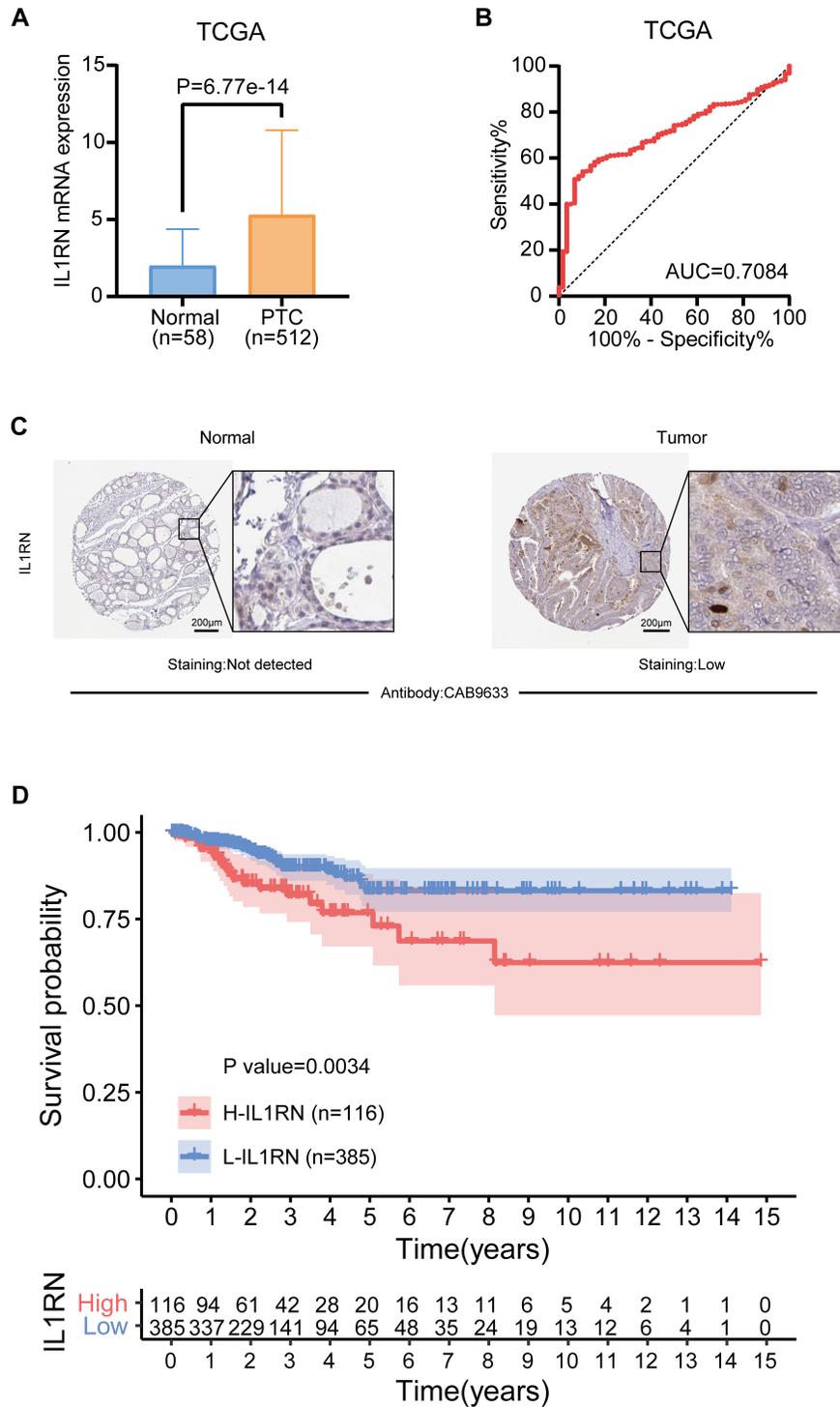


Figure 1

Diagnostic value of IL1RN in PTC. (A) Comparison of IL1RN mRNA expression between PTC and normal tissues based on data from TCGA. (B) Diagnostic efficacy of IL1RN as shown by the ROC curve. (C) Comparison of immunohistochemical images indicating IL1RN expression in PTC and normal thyroid tissues. (D) Kaplan–Meier curve of the progression-free survival (PFS) of the H-IL1RN group and L-IL1RN group. * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$ here and in the following figures.

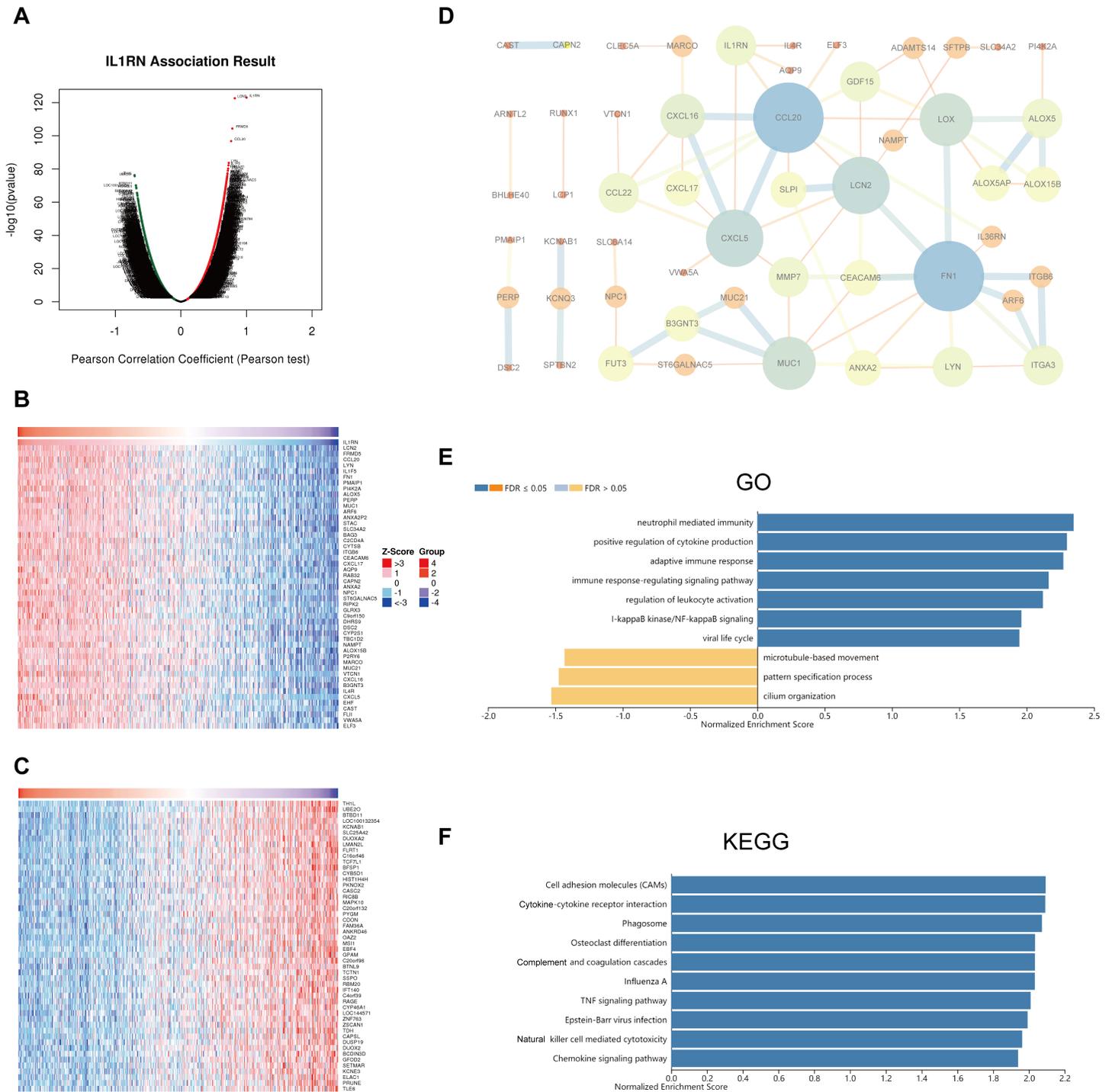


Figure 2

Potential mechanisms of IL1RN in PTC. (A) Genes highly correlated with IL1RN identified by Pearson correlation analysis in the TCGA cohort. (B) Heatmaps showing the top 50 genes positively and (C) negatively correlated with IL1RN in the TCGA cohort. (D) PPI of the top 100 significantly correlated genes. (E) GO biological process terms and (F) KEGG pathways significantly enriched in genes coexpressed with IL1RN in the TCGA cohort.

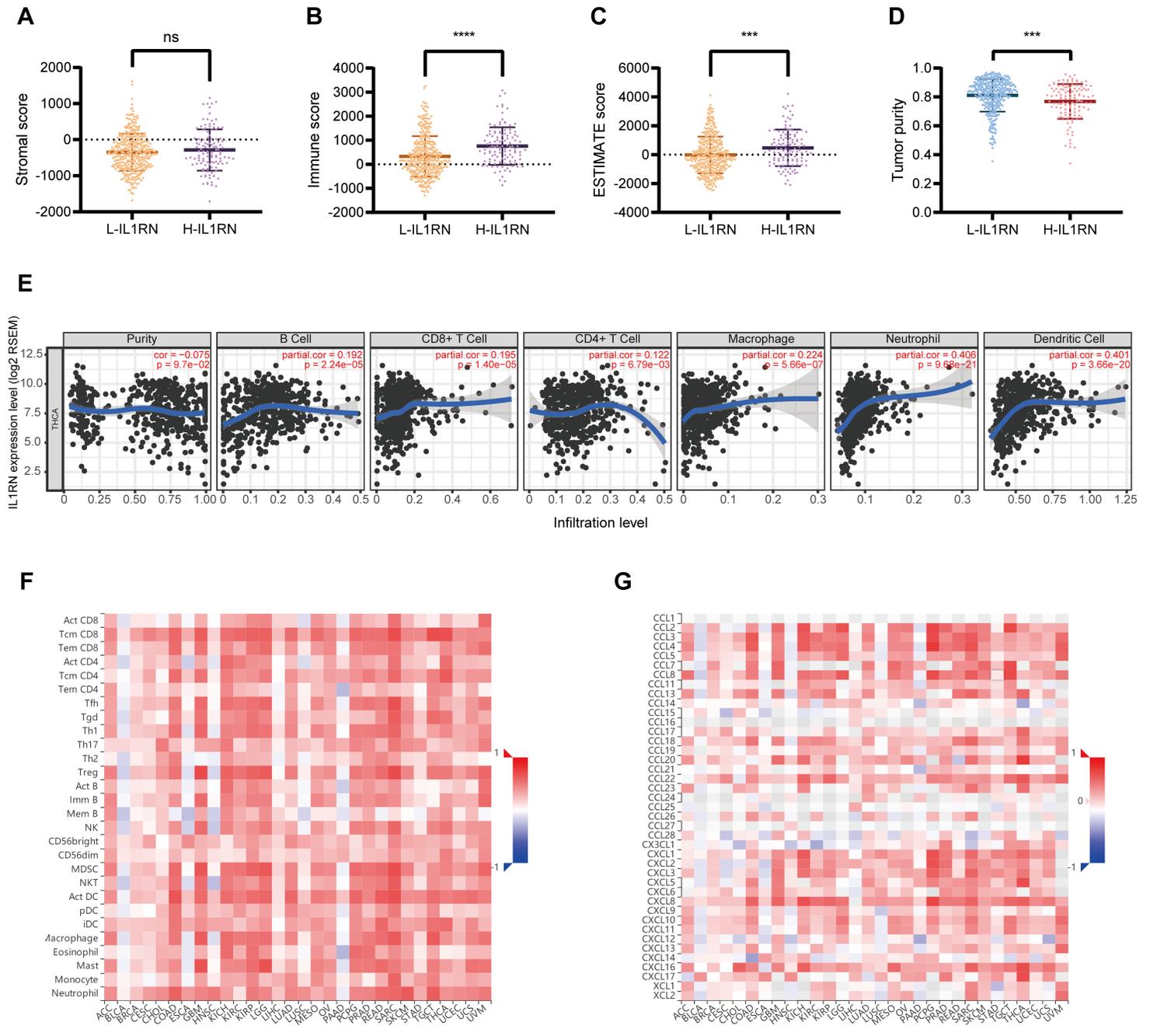


Figure 3

IL1RN is closely related to immunity in PTC. ESTIMATE analysis of (A) stromal scores, (B) immune scores, (C) ESTIMATE scores, and (D) tumor purity in the L-IL1RN and H-IL1RN groups. (E) TIMER analysis of the purity-corrected partial Spearman correlations between the expression of IL1RN and the infiltration of six types of immune cells in the TCGA cohort. (F) Correlation analysis of the expression of IL1RN and 28 types of TILs in various human cancers by TISIDB. (G) Correlation analysis of the expression of IL1RN and the levels of chemokines in various human cancers by TISIDB.

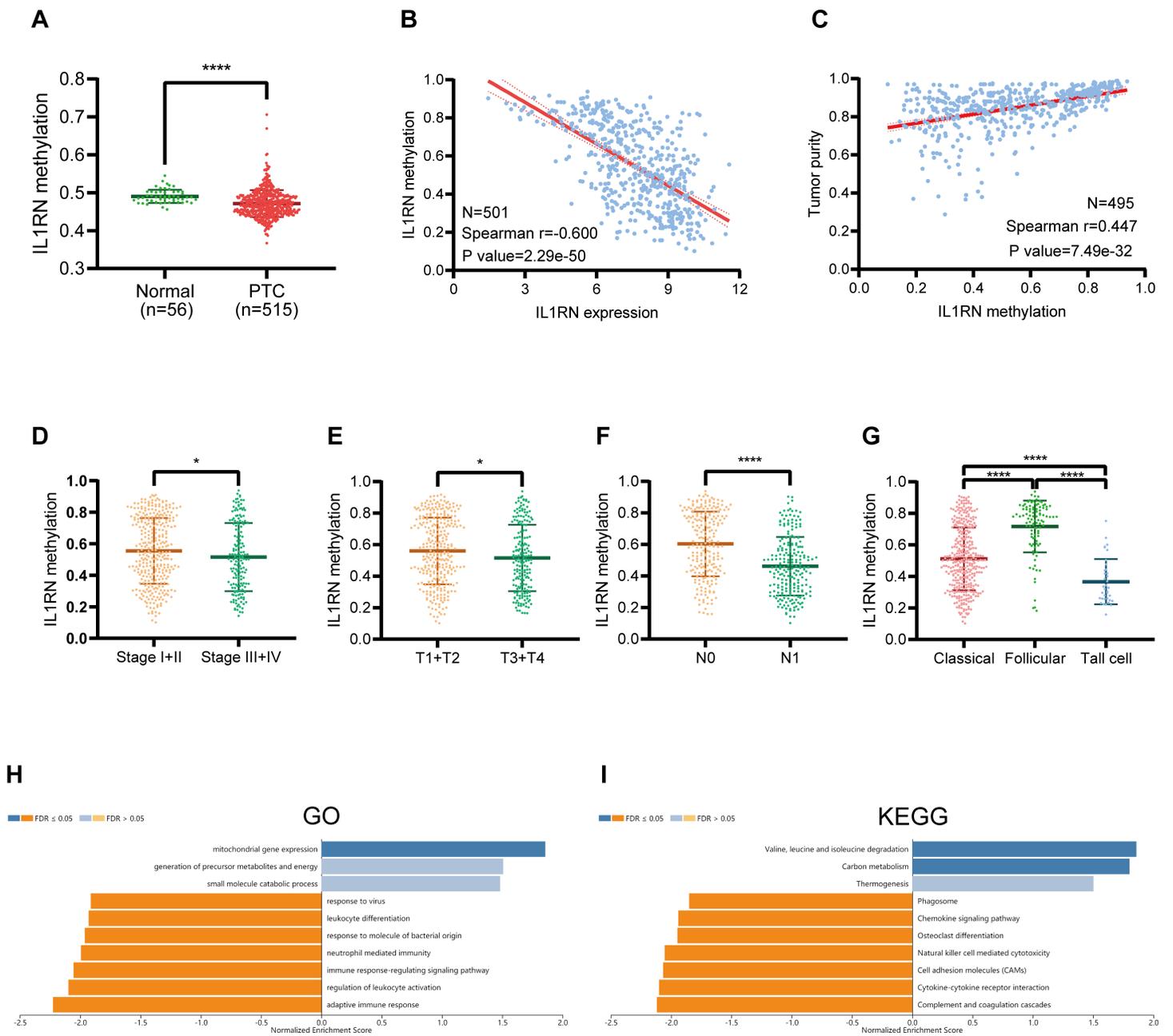


Figure 4

Methylation of IL1RN in PTC. (A) IL1RN methylation levels were compared between PTC and normal tissues. (B) Correlation analysis between IL1RN methylation and IL1RN expression in PTC. (C) Correlation analysis between IL1RN methylation and tumor purity in PTC. IL1RN methylation level among patients with different (D) clinical stages, (E) T classifications, (F) N classifications, and (G) histological types of PTC. (H) GO biological process terms and (I) KEGG pathways significantly altered by IL1RN methylation in the TCGA cohort.

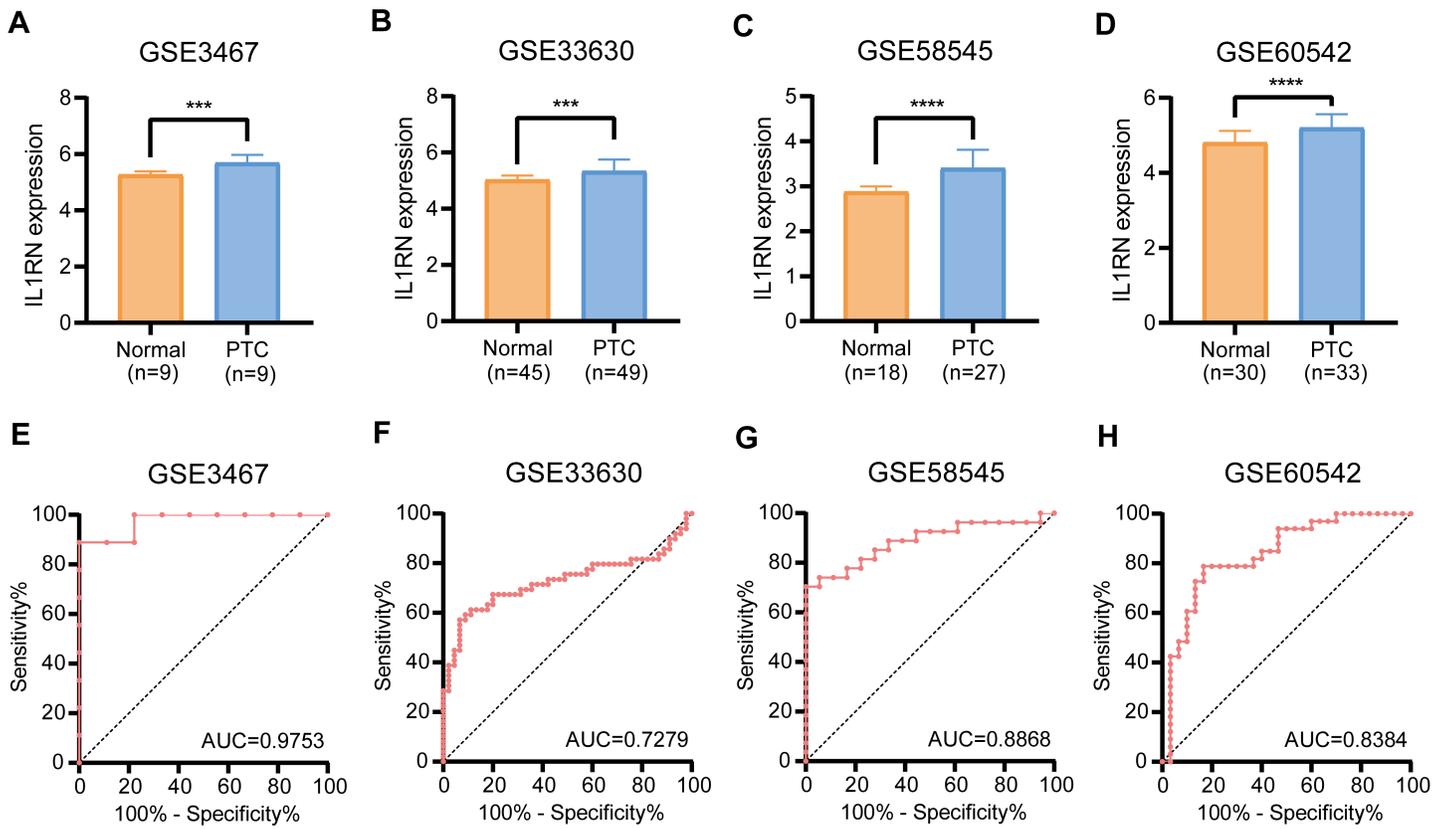


Figure 5

(A-D) mRNA expression of IL1RN in 4 GEO verification cohorts (GSE3467, GSE33630, GSE58545, and GSE60542). (J-O) ROC curve showing the diagnostic value of IL1RN expression in the verification cohorts.

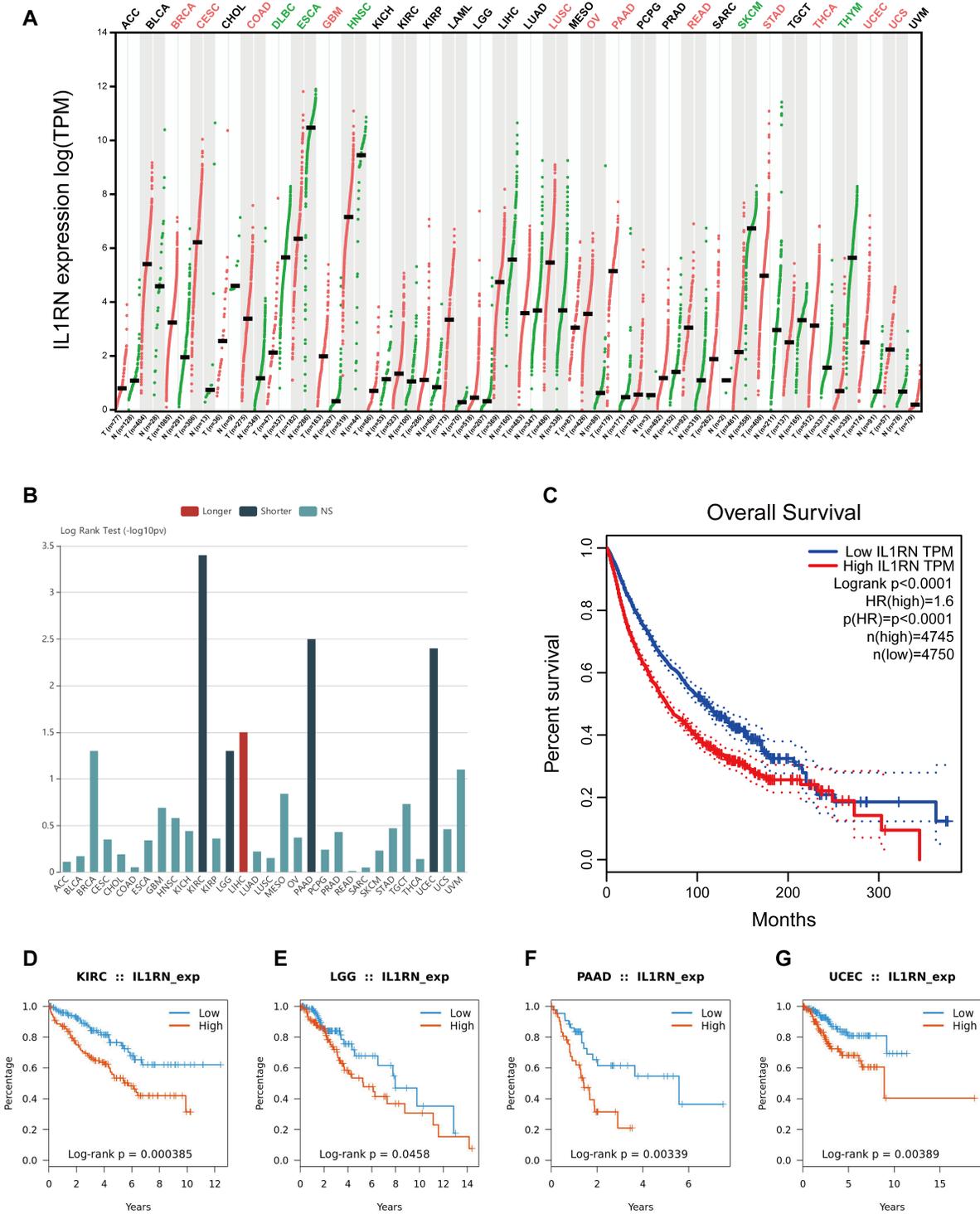


Figure 6

Generalizability of differences in IL1RN expression in different cancers. (A) Comparison of IL1RN mRNA expression between cancer and paracancerous tissues in different cancers. (B) Association between TREM-1 expression and OS in different human cancers. K-M survival analysis between the L-IL1RN and H-IL1RN groups in (C) the pancancer, (D) KIRC, (E) LGG, (F) PAAD, and (G) UCEC cohorts.

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