Expression and prognosis analysis of integrin subunit α3 (ITGA3) in papillary thyroid cancer

Jun-jie Ma [a], Cheng Xiang [a], Heng-qing Zhu [a], Bing-long Bai [a], Ping Wang [a], Guan-an Zhao [b,*]

[a] *Department of Thyroid Surgery, The Second Affiliated Hospital of Zhejiang University School of Medicine, Hangzhou 310009, Zhejiang, China*
[b] *Department of Urology, Lishui City People's Hospital, Lishui 323000, Zhejiang, China*

ARTICLE INFO

ABSTRACT

Integrin subunit α3 (ITGA3) is a member of the integrin family and interacts with extracellular matrix proteins. However, there have been few reports regarding the role of ITGA3 in papillary thyroid cancer. The expression levels of ITGA3 were firstly analyzed by bioinformatics tools and *in vitro* experiments, followed by evaluating its prognostic significance in papillary thyroid cancer patients using Kaplan-Meier, receiver operating characteristic, and Cox regression analyses. Then, cBioportal and GSCA databases were applied to evaluate genetic alterations of ITGA3. Functional enrichment analysis was conducted and the upstream miRNAs of ITGA3 were determined. The results showed that the ITGA3 mRNA and protein levels were higher in the papillary thyroid cancer group than those in the normal group (all $P < 0.05$). Moreover, ITGA3 performed well in distinguishing the recurrence-free survival (RFS) status and served as an independent prognostic factor of papillary thyroid cancer patients ($P < 0.01$). Besides, significant relations between ITGA3 and genetic alterations were observed (FDR <0.01). Functional enrichment analysis indicated ECM-receptor interaction and cell adhesion molecules were the shared regulatory pathways. Moreover, ITGA3 might be the target gene of hsa-miR-3129, hsa-miR-181d, hsa-miR-181b, hsa-miR-199a, and hsa-miR-199b. Of note, the ITGA3 mRNA level was reduced after has-miR-199b-3p/5p was overexpressed. In conclusion, ITGA3 could be a reliable biomarker and have potential value in predicting the RFS status of papillary thyroid cancer patients.

1. Introduction

Thyroid cancer is the most common endocrine malignancy, which was secondary to cancers of breast, lung, rectal, and cervical among female malignancies in 2017 [1,2]. In China, there were approximately 90,000 thyroid cancer cases in 2015 [3]. The number of new thyroid cancer cases reached 12.9 per 100,000 populations annually in 2015 in the United States [4]. According to the statistics published by the American Cancer Society, about 12,150 and 32,130 new thyroid cancer cases occurred in males and females, respectively in 2021 [5]. It can be classified into papillary thyroid, follicular thyroid, medullary thyroid, and undifferentiated thyroid cancer types. Among these, papillary thyroid cancer accounts for 85 % of all thyroid cancers [6]. The incidence of papillary thyroid cancer has been increasing due to advancements in diagnostic techniques including fine-needle biopsy and ultrasound [7]. Despite the

* Corresponding author. Department of Urology, Lishui City People's Hospital, No.15, Dazhong Street, Liandu District, Lishui 323000, Zhejiang, China.
E-mail address: lyzhaoguanan@163.com (G.-a. Zhao).

majority of papillary thyroid cancer patients having a good prognosis, recurrences are observed in nearly 30 % of the patients [8]. Therefore, it is urgent to develop novel biomarkers with good sensitivity and specificity to improve the diagnosis and prognosis of papillary thyroid cancer patients.

Integrins are cell surface receptors for extracellular matrix (ECM) proteins, which are widely expressed in tissues and organs in the human body [9]. Integrins are transmembrane heterodimers composed of two noncovalently associated glycoprotein α and β subunits [10]. They represent a crucial role in the proliferation, migration, invasion, and metastasis of cancer cells [11,12]. As a member of the integrin family, integrin subunit α3 (ITGA3) undergoes posttranslational cleavage in the extracellular domain to yield disulfide-linked light and heavy chains to join with a β1 subunit, forming an intact integrin α3β1. As a main receptor linking epithelial cells to basement membranes, integrin α3β1 interacts with ECM proteins, mediating cell-cell adhesion and cell-matrix adhesion, and connecting the external and internal structures of cells [13]. ITGA3 is widely expressed in normal organisms, but under the effects of oncogene induction, alterations in the chromatin structure, overexpression of growth factors and its receptor, and changes in the ECM cause an aberrant expression that induces cancer [10]. Huang et al. showed that ITGA3 was highly expressed in intrahepatic cholangiocarcinoma, promoting cell proliferation and cell cycle progression [14]. The elevated levels of ITGA3 in head and neck cancer led to an unfavorable prognosis for the patients [15]. In addition, ITGA3 was directly regulated by miR-223, and silencing of ITGA3 significantly inhibited prostate cancer cell migration and invasion [16]. However, there have been few reports regarding the role of ITGA3 in thyroid cancer.

This study aimed to explore the relationship between ITGA3 expression and the prognosis of papillary thyroid cancer patients from the following aspects: the expression levels of ITGA3 were first analyzed using efficient bioinformatics tools and *in vitro* experiments. Then, the prognostic significance of ITGA3 mRNA expression was evaluated. Enrichment analyses were conducted, and the upstream miRNAs of ITGA3 were identified to reveal the underlying molecular mechanisms of abnormal ITGA3 in papillary thyroid cancer.

2. Materials and methods

2.1. Expression analysis of ITGA3

We first explored the mRNA expression of ITGA3 in pan-cancers through Gene Set Cancer Analysis (GSCA) database (http://bioinfo.life.hust.edu.cn/GSCA/#/). Next, RNA-seq-HTseq-FPKM data for papillary thyroid cancer samples based on GDC-TCGA were collected from UCSC Xena (https://xenabrowser.net/datapages/) database. These data were used to assess the ITGA3 mRNA expression in papillary thyroid cancer and normal thyroid tissues using unpaired and paired t-tests. Then, RNA-seq expression and clinical information based on TCGA were downloaded from the cBioPortal database. We used the TCGA-THCA data composed mainly of papillary thyroid cancer samples to determine the association of ITGA3 mRNA expression with clinical variables. Moreover, the mRNA expression of ITGA3 as a continuous variable in papillary thyroid cancer based on clinical characteristics including age, gender, cancer stage, and histological subtype was analyzed via the UALCAN database (http://ualcan.path.uab.edu/). UALCAN is a comprehensive, user-friendly, and interactive web resource for analyzing cancer omics data.

2.2. Cell lines and cell culture

To verify the expression of ITGA3 in papillary thyroid cancer, normal cell line Nthy-ori 3-1 and papillary thyroid cancer cell line MDA-T32 were obtained from ATCC. These cell lines were cultured in Dulbecco's modified Eagle's medium (Gibco, USA) with 10 % fetal bovine serum (Gibco, USA) under the atmosphere at 37 °C with 5 % CO_2.

2.3. Quantitative real-time polymerase chain reaction (qRT-PCR) analysis

Total RNA was isolated from cells using TRIzol reagent (Invitrogen, USA) following the manufacturer's protocol. Takara Prime-Script RT reagent kit (Takara, Japan) was used to synthesize the first-strand cDNA. Next, the SYBR Premix Ex Taq (Takara, Japan) was used to perform the qRT-PCR assay according to the manufacturer's instructions. The sequences of primers were used as ITGA3 Forward primer 5′-TCAACCTGGATACCCGATTCC-3′, Reverse primer 5′-GCTCTGTCTGCCGATGGAG-3′; GAPDH Forward primer 5′-GTCTCCTCTGACTTCAACAGCG-3′, Reverse primer 5′-ACCACCCTGTTGCTGTAGCCAA-3′. GAPDH was considered to be a control for relative quantification. The comparative cycle threshold ($2^{-\Delta\Delta CT}$) method was used for the calculation of relative mRNA expression.

2.4. Western blot analysis

Total protein was extracted from the indicated cells with RIPA buffer containing protease inhibitors (Beyotime, China). The Western blot analysis was performed as previously described [17]. The primary antibodies included in this study were anti-ITGA3 (1:1000, HA500111, HUABIO) and anti-GAPDH (1:2000, ab125247, abcam). An HRP-conjugated antibody was used as the secondary antibody. The bands were visualized by the enhanced chemiluminescence method. Image J software was used to quantify the protein levels.

2.5. Survival analysis of ITGA3

Using the Kaplan-Meier plotter website (http://kmplot.com/), we examined the influence of ITGA3 on the overall survival (OS) and

the recurrence-free survival (RFS) of papillary thyroid cancer patients by splitting patients with the best cutoff [18]. We used "survfit" in the R package to analyze the association of ITGA3 expression with clinical outcomes according to age ($<55/\geq55$), gender (female/male), and stage ($1+2/3+4$). Maxstat statistics was adopted to determine the optimal cutoff for dividing high and low ITGA3 expression samples. Then, the value of ITGA3 in distinguishing the survival status of papillary thyroid cancer patients was analyzed by calculating the area under the curve (AUC) of the ROC curves. Further, univariate and multivariate Cox regression analyses were performed to predict the independent prognostic factors for RFS in papillary thyroid cancer patients. The female was set as a reference level for gender, stage $1+2$ for stage, and Asian for race.

2.6. Mutation, copy number variation (CNV), and methylation analysis of ITGA3

We next focused on the potential mechanism of dysregulated ITGA3 expression and investigated the ITGA3 mutations in papillary thyroid cancer through cBioPortal for Cancer Genomics in the "Mutations" column with a total of 397 samples. In addition, the
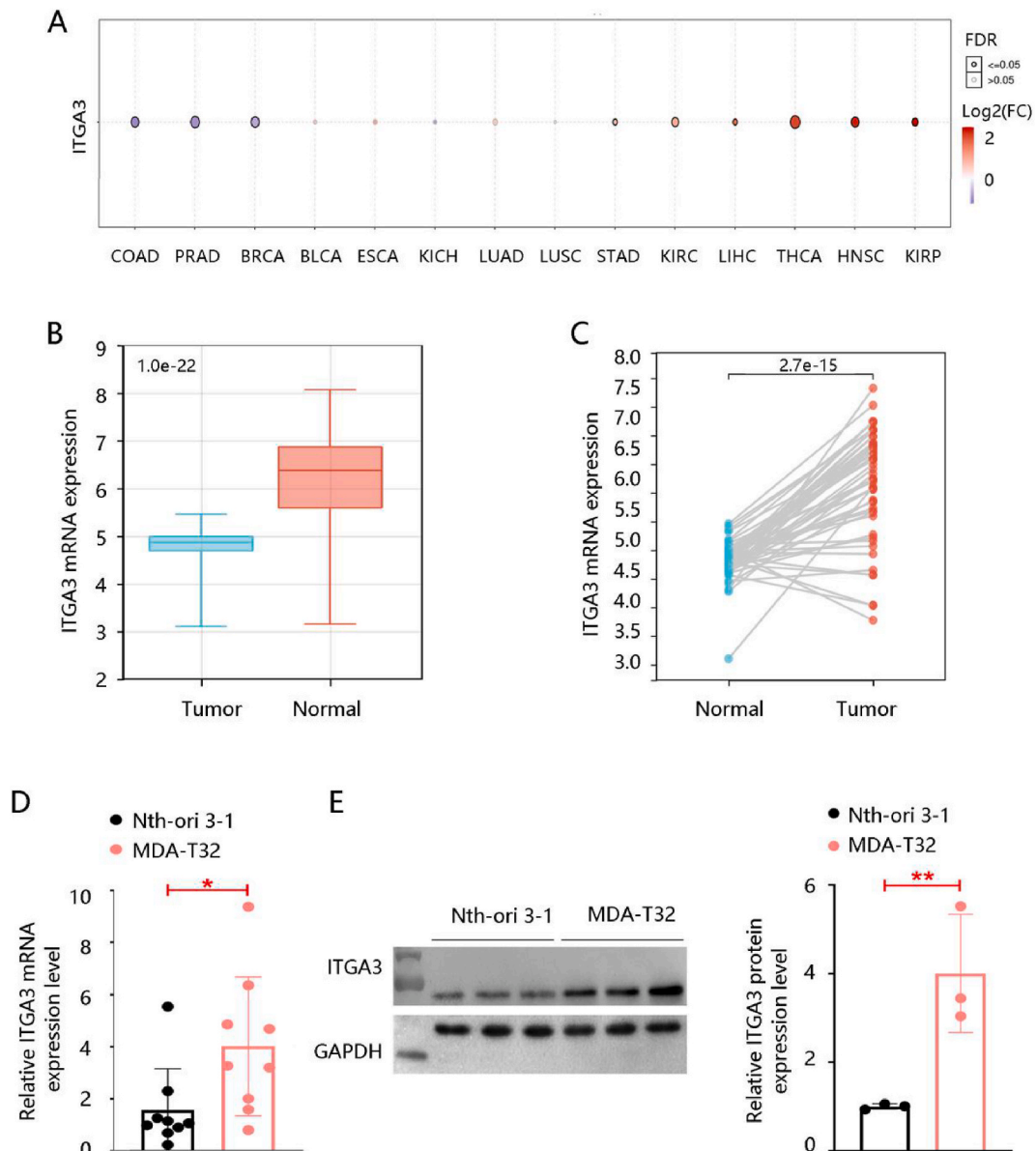

Fig. 1. The gene and protein expression of ITGA3. **(A)** ITGA3 mRNA expression in various cancers. The differential mRNA expression of ITGA3 in papillary thyroid cancer and normal tissues using **(B)** unpaired and **(C)** paired t-tests. **(D)** qRT-PCR analysis of relative ITGA3 mRNA expression in normal Nthy-ori 3-1 cell line and papillary thyroid cancer cell line MDA-T32. **(E)** Western blot analysis of relative ITGA3 protein expression in the two cell lines. *P < 0.05, **P < 0.01.

association of ITGA3 mRNA expression with CNV, and DNA methylation were investigated, respectively through the GSCA database. GSCA is an integrated database for genomic and immunogenomic gene set cancer analysis.

2.7. Enrichment analysis

The R software limma package was employed to identify the DEGs between the low and high ITGA3 expression groups (cutoff value of 50 %) in gene expression profiles of papillary thyroid cancer. An absolute log2FC > 1 and *P*-value <0.05 were used as the filter criterion for significant DEGs. The significantly enriched gene ontology (GO) terms and the kyoto encyclopedia of genes and genomes (KEGG) pathways of these DEGs were investigated using the clusterProfiler in the R package. *P*-value <0.05 and false discovery rate (FDR) < 0.25 were considered statistically significant.

Subsequently, GSEA was performed to elucidate the survival differences between the low and high ITGA3 expression groups. The gene set was permutated 1000 times and the expression level of ITGA3 was used as a phenotypic label. A nominal p-value <0.05 and an FDR q-value <0.25 were considered to be statistically significant.

2.8. Identification of upstream miRNAs

To further understand the underlying mechanism of papillary thyroid cancer, we predicted the target upstream miRNAs of ITGA3. The targeted miRNAs of ITGA3 were explored by DINAN (http://diana.imis.athena-innovation.gr/DianaTools/index.php), miRDB (http://www.mirdb.org/), miRWALK (http://mirwalk.umm.uni-heidelberg.de/), and Targetscan (http://www.targetscan.org/vert_71/) databases. Then, the consistent miRNAs in four databases were screened by Venn analysis (http://bioinformatics.psb.ugent.be/webtools/Venn/). The influence of the identified miRNAs on the prognosis of papillary thyroid cancer patients was evaluated via the Kaplan-Meier plotter website.

2.9. Dual-luciferase reporter experiment

The sequence of ITGA3 and has-miR-199b-3p/miR-199b-5p was amplified using PCR. The amplification product was inserted into the empty luciferase reporter vector (Promega, Madison, WI, USA) to establish ITGA3 3′UTR wild-type plasmids, and the binding fragment was mutated using the gene mutation technique to construct ITGA3 3′UTR mutant plasmids. The recombinant plasmids were co-transfected into 293 T cells with either mimic miRNAs or negative control, respectively. After 48h, the luciferase activity of the cells was measured and normalized to the activity of Rluc.

2.10. Statistical analysis

All statistical analyses were performed by SPSS 23.0 (SPSS, Inc., Chicago, IL, USA) and R software. The association of ITGA3 levels with clinicopathological factors was qualitatively analyzed using the chi-squared test. Kaplan-Meier plotter curves and the log-rank test were performed to determine survival differences. The relationship between ITGA3 and CNV and DNA methylation was analyzed by the Spearman correlation test in GSCA. The Pearson correlation test was utilized to evaluate the relationship between ITGA3 expression and miRNAs in starBase. P < 0.05 was considered statistically significant.

Table 1
The relationship between ITGA3 mRNA expression and clinical variables in papillary thyroid cancer.

Characteristics	Low ITGA3 expression (%)	High ITGA3 expression (%)	*P*-value
Age			0.695
<55	120 (70.2)	124 (72.1)	
≥55	51 (29.8)	48 (27.9)	
Gender			0.926
Male	128 (74.9)	128 (74.4)	
Female	43 (25.1)	44 (25.6)	
Stage			0.018
1	105 (61.4)	100 (58.5)	
2	23 (13.5)	12 (7.0)	
3	33 (19.3)	34 (19.9)	
4	10 (5.8)	25 (14.6)	
Histological subtype			<0.001
PTC-classical	104 (60.8)	144 (83.7)	
PTC-follicular	65 (38.0)	6 (3.5)	
PTC-tall cell	1 (0.6)	20 (11.6)	

Abbreviations: PTC, papillary thyroid cancer.

3. Results

3.1. Expression analysis of ITGA3

To evaluate the expression of ITGA3 in papillary thyroid cancer, we first visualized the ITGA3 mRNA expression in various cancers through the GSCA database. As shown in Fig. 1A, ITGA3 was highly expressed in 9 types of cancer including thyroid cancer (all P < 0.05). Quantitative analysis confirmed ITGA3 overexpression in papillary thyroid cancer compared with normal thyroid tissue (P < 0.05) (Fig. 1B–C). Next, we compared the relative mRNA and protein expression of ITGA3 in normal thyroid cell line Nthy-ori 3-1, and papillary thyroid cancer cell line MDA-T32 using qRT-PCR and western blot, respectively. Both mRNA and protein levels of ITGA3 were higher in the MDA-T32 cell than those in Nthy-ori 3-1 cell (P < 0.05) (Fig. 1D–E).

Further, the relationship between ITGA3 mRNA expression and clinical variables was qualitatively analyzed. As shown in Table 1, ITGA3 expression was significantly associated with stage and histological subtype (P < 0.05) but had no significant impact on age and gender. The same results were observed in quantitative analysis (Fig. 2A–D). Notably, patients at stage 4 tended to have the highest ITGA3 mRNA expression (Fig. 2C); and papillary thyroid cancer-tall cell had significantly higher ITGA3 expression than papillary thyroid cancer-classical and papillary thyroid cancer-follicular (Fig. 2D).

3.2. Survival analysis of ITGA3

To investigate the effect of ITGA3 on OS and RFS in papillary thyroid cancer patients, the survival analysis was carried out using a Kaplan-Meier plotter. As exhibited in Fig. 3A, ITGA3 had no significant impact on OS (P > 0.05). However, patients with higher ITGA3 mRNA expression had shorter RFS with a hazard ratio of 3.09 (P < 0.01) (Fig. 3B). Further stratified analysis showed that high ITGA3 expression correlated with poor RFS in all the subgroups **(Table 2)**. The ROC curve result showed that the entire AUC was 0.754 (95 % CI: 0.652–0.855) (Fig. 4A). After considering the time factor, we found that ITGA3 still had satisfactory performance in predicting the RFS status of papillaty thyroid cancer patients (the AUCs of 1-, 3-, and 5-year were 0.78, 0.77, and 0.72, respectively) (Fig. 4B). These findings revealed the potential value of ITGA3 in predicting the survival status of papillary thyroid cancer patients.
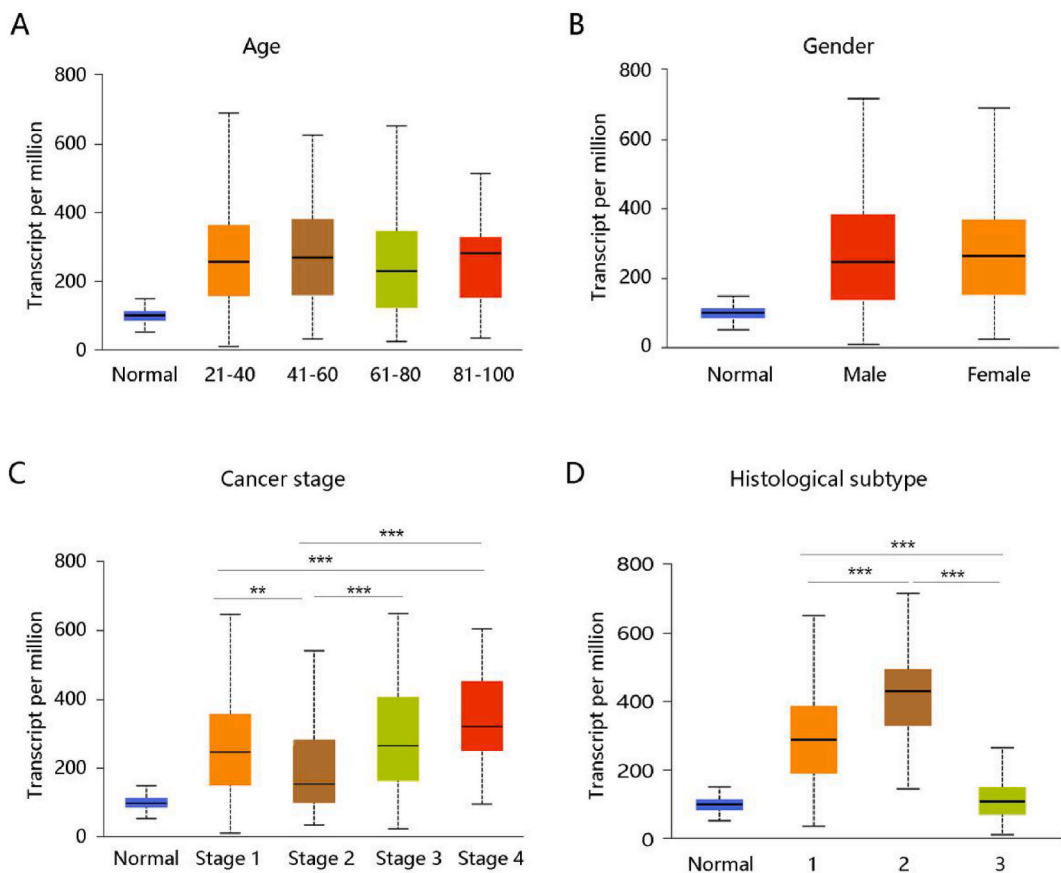

Fig. 2. The mRNA expression of ITGA3 in papillary thyroid cancer based on clinical characteristics. **(A)** Age. **(B)** Gender. **(C)** Cancer stage. **(D)** Histological subtype. 1, papillary thyroid cancer-classical; 2, papillary thyroid cancer-tall cell; 3, papillary thyroid cancer-follicular. The ITGA3 mRNA expression in all tumor groups was significantly different from the normal group. **P < 0.01, ***P < 0.001.
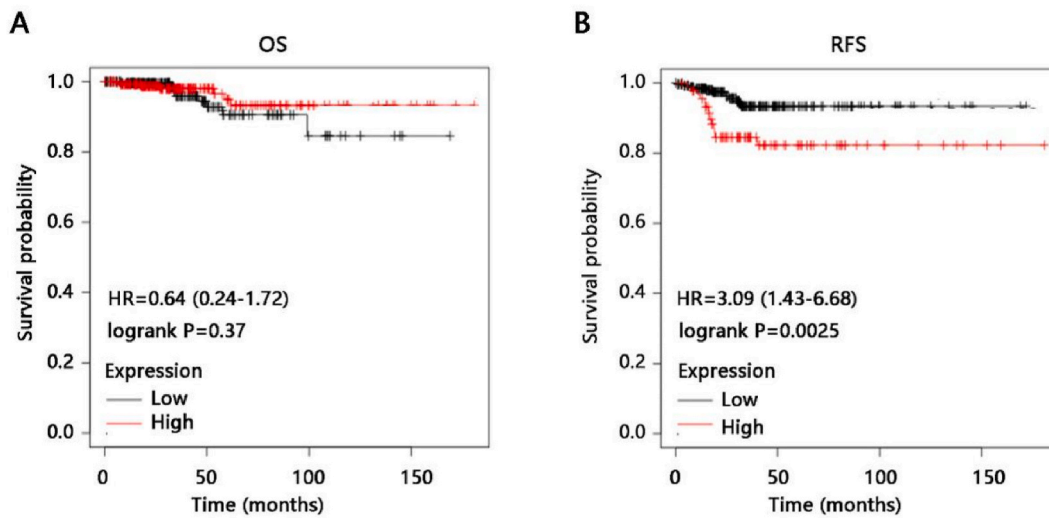
Fig. 3. The effect of ITGA3 on overall survival and recurrence-free survival in papillary thyroid cancer. **(A)** OS. **(B)** RFS. OS, overall survival; RFS, recurrence-free survival; HR, hazard ratio.

Table 2
The correlation between ITGA3 mRNA expression and recurrence-free survival in papillary thyroid cancer patients with restricted pathological factors.

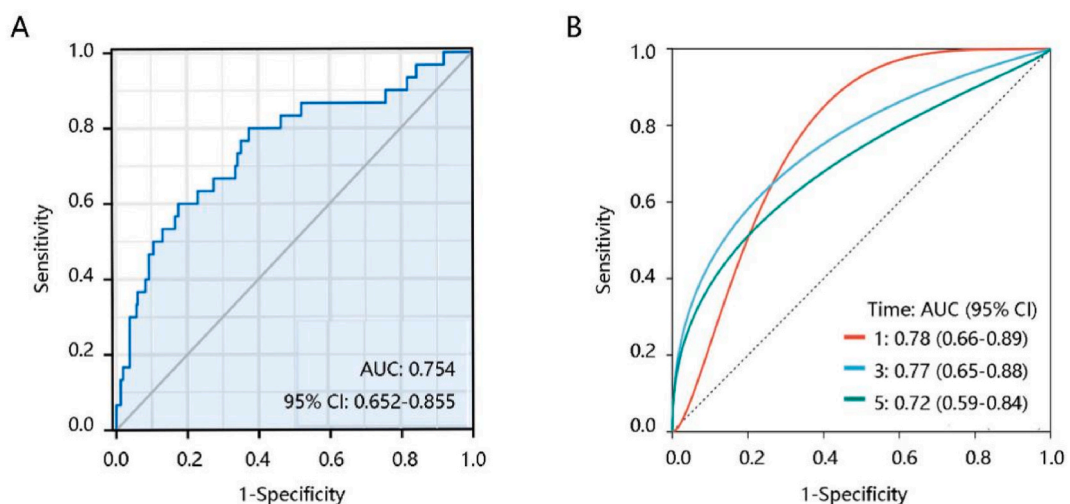| Clinicopathological parameters	Number	Hazard ratio (95 % CI)	*P*-value
Age			
<55	244	6.62 (2.33–18.79)	4.2e-5
≥55	99	6.02 (1.65–21.94)	2.0e-3
Gender			
Female	256	6.32 (2.14–18.69)	1.3e-4
Male	87	8.96 (1.81–44.44)	1.1e-3
Stage			
Stage 1 + 2	240	6.05 (1.86–19.64)	6.4e-4
Stage 3 + 4	102	4.88 (1.84–12.95)	4.4e-4

Abbreviations: CI, confidence interval.

Fig. 4. The value of ITGA3 in predicting recurrence-free survival status of papillary thyroid cancer patients. **(A)** The receive operating characteristic (ROC) analysis in papillary thyroid cancer patients. **(B)** Time-dependent ROC analysis in papillary thyroid cancer patients. AUC, area under the curve; 95 % CI, 95 % confidence interval.

After that, univariate Cox regression analysis was used to evaluate the factors influencing RFS, showing that stage (3 + 4 vs. 1 + 2, P < 0.001), and ITGA3 (P < 0.001) were predictive factors for poor RFS (Table 3). Nevertheless, other clinical factors including age, gender, African-American, and Caucasian were not significantly related to patient prognosis. After integrating these factors into multivariate Cox regression analysis, stage 3 + 4 (P = 0.027) and high expression of ITGA3 (P = 0.002) were still independent prognostic factors for worse RFS (Table 3). Therefore, high expression of ITGA3 could independently predict worse RFS among papillary thyroid cancer patients.

3.3. Mutation, CNV, and methylation analyses of ITGA3

Due to the independent prognostic value of ITGA3 for RFS in papillary thyroid cancer patients, we further investigated the potential mechanism of dysregulated ITGA3. A mutation plot from the cBioportal for Cancer Genomics presented that the mutation sites of ITGA3 were D790 N, and R874L with mutation frequency of 0.25 % each (Fig. 5A). Besides, the mRNA expression of ITGA3 showed a significantly negative correlation with CNV (Cor. = -0.16, FDR = 3.1e-03) (Fig. 5B). Similarly, the mRNA expression was negatively related to DNA methylation (Cor. = -0.42, FDR <0.001) (Fig. 5C). Therefore, the mutations, CNV, and DNA methylation might lead to the aberrant upregulation of ITGA3 in papillary thyroid cancer.

3.4. Functional enrichment analysis

To elucidate the pathological role of ITGA3 in papillary thyroid cancer, the GO and KEGG analyses of the DEGs between the low and high ITGA3 expression groups were performed using the clusterProfile package. A total of 596 DEGs were generated from gene expression RNA-seq-HTseq-FPKM, including 198 upregulated and 398 downregulated DEGs as shown in the volcano plot (Fig. 6A) and the heat map (Fig. 6B). Functional enrichment analysis results presented that the major GO terms were biological adhesion, cell adhesion, extracellular region, vesicle, signal receptor binding, and MHC class II receptor activity (Fig. 6C). As for the KEGG pathway, they were mainly enriched in PI3K-Akt, cell adhesion molecules, MAPK signaling pathway, and ECM-receptor interaction (Fig. 6D).

Then, GSEA was conducted to gain further insight into the biological pathways involved in papillary thyroid cancer. Among the top 5 pathways in high ITGA3 expression phenotype, ECM-receptor interaction, and cell adhesion molecules were the consistent pathways with KEGG analysis (Fig. 7). Thus, high expression of ITGA3 contributed to the unfavorable clinical outcomes of papillary thyroid cancer patients, which might be related to the activation of these pathways, especially ECM-receptor interaction, and cell adhesion molecules.

3.5. Upstream miRNA identification of ITGA3

To further reveal the underlying mechanism of papillary thyroid cancer, we looked for the potential upstream regulators of ITGA3 from various databases. There were 97, 102, 31765, and 12 targeted miRNAs detected in DINAN, miRDB, miRWALK, and Targetscan databases, respectively. Totally, 5 consistent miRNAs including hsa-miR-3129, hsa-miR-181d, hsa-miR-181b, hsa-miR-199a, and hsa-miR-199b were obtained through Venn analysis (Fig. 8A). The Kaplan-Meier plotter analysis showed that high expression levels of hsa-miR-181b, hsa-miR-181d, and hsa-miR-3129 led to a favorable prognosis (all P < 0.05) (Fig. 8B–D). Nevertheless, the patients with high hsa-miR-199b and hsa-miR-199a had a shorter survival time (all P < 0.001) (Fig. 8E–F).

Next, we chose hsa-miR-199b of interest for validation (Fig. 9A). After the wide type and mutated type of luciferase reporters were constructed, a dual-luciferase reporter gene experiment was performed. The results showed that hsa-miR-199b-3p and hsa-miR-199b-5p significantly repressed the luciferase activity of the wild-type reporter, but not that of the mutated reporter (Fig. 9B). This indicated the has-miR-199b combined with the ITGA3.

Following this, the hsa-miR-199b-3p mimics and hsa-miR-199b-5p mimics were transfected to the MDA-T32 cell line. The expression levels of miR-199b-3p/5p were dramatically increased after transfection (P < 0.01) (Fig. 9C). Then, we analyzed the relative ITGA3 mRNA expression level in the MDA-T32 cell after hsa-miR-199b-3p and hsa-miR-199b-5p were overexpressed. The qRT-PCR results showed that the relative mRNA levels of ITGA3 were notably decreased in the hsa-miR-199b-3p/5p overexpressed groups compared with the NC group (Fig. 9D). The western blot analysis exhibited that the protein levels of ITGA3 were notably reduced in the MDA-T32 cell upon transfection of hsa-miR-199b-3p/5p mimics in contrast to the NC group (Fig. 9E). Subsequently, we

Table 3
Cox regression analysis of factors associated with recurrence-free survival in papillary thyroid cancer.

Characteristics	Univariate analysis		Multivariate analysis	
	HR (95 % CI)	P-value	HR (95 % CI)	P-value
Age	1.016 (0.992–1.040)	0.197	0.991 (0.953–1.030)	0.646
Gender (Male vs. Female)	1.208 (0.537–2.720)	0.648	0.912 (0.347–2.395)	0.851
Stage (3 + 4 vs. 1 + 2)	3.687 (1.790–7.597)	<0.001	3.428 (1.154–10.182)	0.027
African-American	0.030 (0.000–15975.103)	0.602	0.000 (0.000-Inf)	0.982
Caucasian	1.625 (0.384–6.872)	0.509	1.541 (0.358–6.636)	0.562
ITGA3	1.011 (1.006–1.012)	<0.001	1.002 (1.001–1.003)	0.002

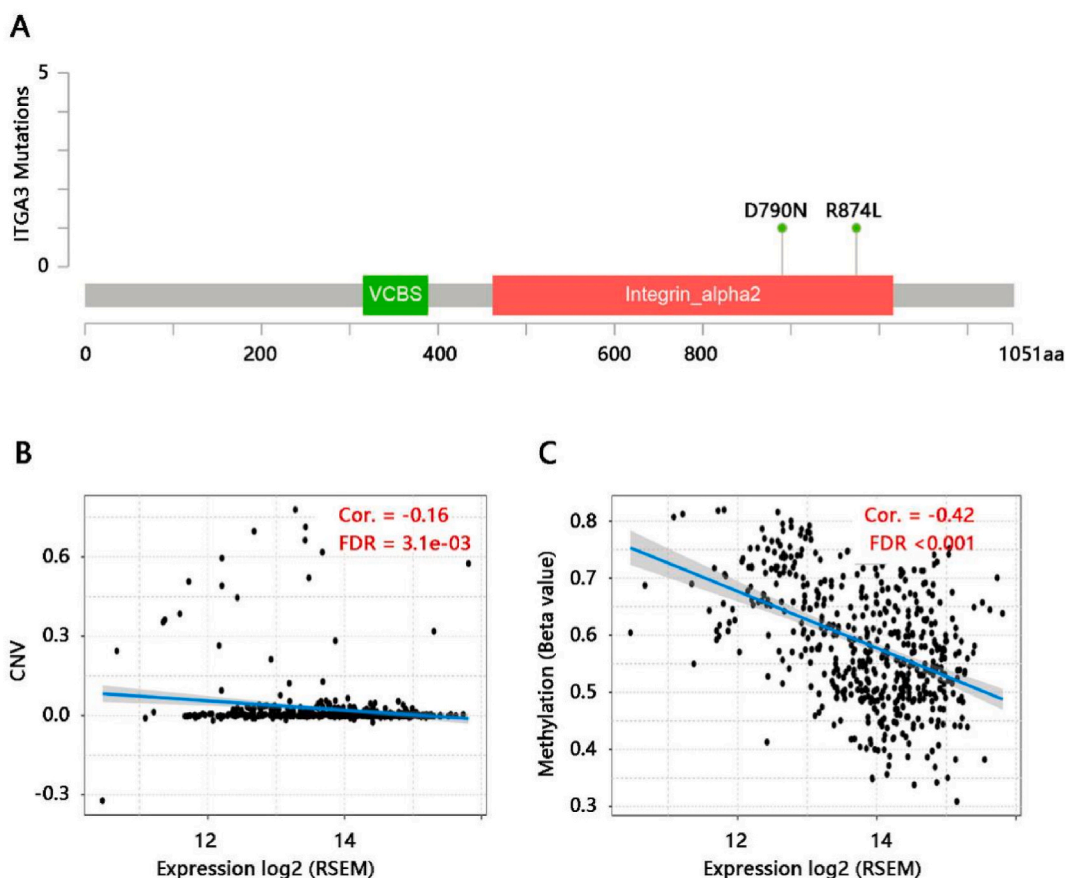Abbreviations: HR, hazard ratio; CI, confidence interval.

Fig. 5. Mutation, copy number variation, and methylation of ITGA3 in papillary thyroid cancer. **(A)** The mutation of ITGA3. **(B)** The relationship between ITGA3 mRNA expression and CNV. **(C)** The relationship between ITGA3 mRNA expression and methylation. CNV, copy number variation; FDR, false discovery rate.

investigated the prognostic value of the combination of has-miR-199b with ITGA3. Although the overall survival difference was not statistically significant (P > 0.05), patients in the high miR-199b + high ITGA3 group had significantly lower survival than those in the high miR-199b + low ITGA3 group (P < 0.05). Besides, the high miR-199b + low ITGA3 group tended to present the longest survival, followed by low miR-199b + low ITGA3 group, low miR-199b + high ITGA3 group, and high miR-199b + high ITGA3 group (Fig. S1). These indicated that the effect of miR-199b inducing prognostic changes by regulating ITGA3 expression is greater than itself leading to prognostic changes directly.

4. Discussion

In this study, ITGA3 was highly expressed at both gene and protein levels in papillary thyroid cancer. ITGA3 mRNA expression was significantly related to the cancer stage and histological subtype. It's worth noting that the high expression of ITGA3 had an intimate relation with worse RFS. Of note, ITGA3 overexpression predicted poor clinical outcomes in all subgroups. Thus, ITGA3 might be a novel and robust prognostic biomarker for papillary thyroid cancer.

Through Cox regression analysis, the upregulated expression of ITGA3 and stage served as independent prognostic factors for worse RFS of papillary thyroid cancer patients. However, age and gender could not independently predict the RFS. Previous studies have demonstrated that advanced stage, elder age, female gender, tall cell type, and residual tumor led to higher rates of all-cause mortality and recurrence [19–21]. Age is related to variations in the expression of the sodium-iodine symporter, which represents a critical role in radioiodine uptake [22]. Thus, the authors speculated that papillary thyroid cancer patients with elder age had higher recurrence rates by affecting the response to therapy. Besides, patients with papillary thyroid cancer-tall cell exhibit more aggressive features such as lymph node metastases, extrathyroidal extension, advanced tumor stage, recurrence, and unfavorable prognosis [23]. The differences between our study and previous studies might be due to analyses in the different cohorts. Further investigations are required in our own cohorts in the future to verify relevant findings.

Tumor-initiating genetic events have become a primary focus in thyroid cancer initiation in the recent decade [24,25]. DNA methylation occurs at CpG sites and CpG exists in two forms: one is dispersed in the DNA sequence; the other is found in a highly
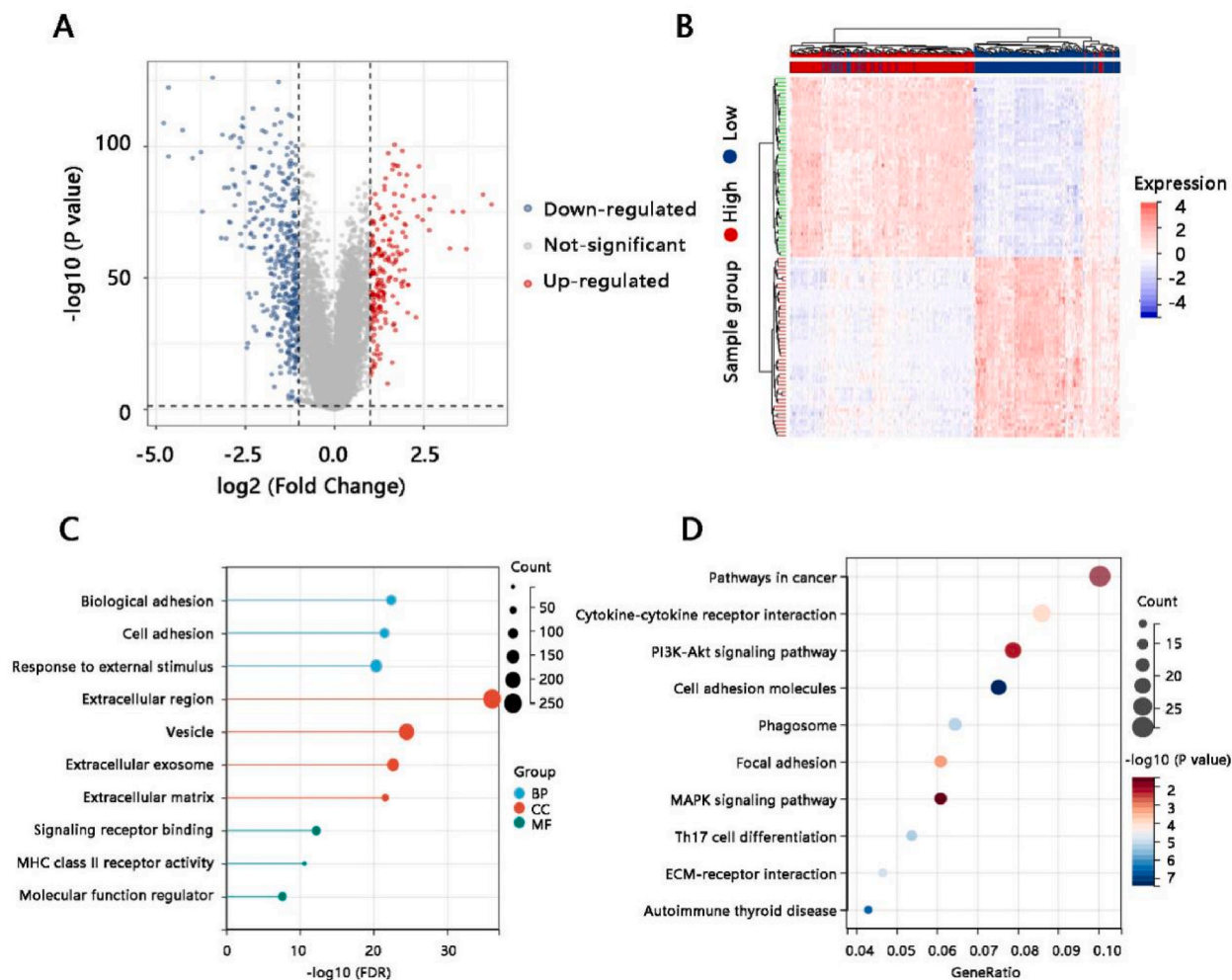
Fig. 6. Functional enrichment analysis of differentially expressed genes (DEGs). **(A)** The volcano plot and **(B)** the heat map of the DEGs. **(C)** Gene ontology and **(D)** Kyoto Encyclopedia of Genes and Genomes pathway enrichment analyses of these DEGs. BP, biological process; CC; cellular component; MF, molecular function.

aggregated state, known as a CpG island. DNA methylation-associated gene silencing underlies many metastasis-related gene expression changes. Additionally, mutations of many genes such as BRCA1 and ERBB2 have been shown to be correlated with an increased risk of metastasis [26]. By analyzing the TCGA data, we found that mutations, CNV, and methylation might result in the aberrant elevation of ITGA3 expression in papillary thyroid cancer, which might be involved in papillary thyroid cancer metastasis. In addition, GO and KEGG analysis of DEGs based on ITGA3 expression levels showed that ITGA3 regulated the development of thyroid cancer by participating in multiple pathways including PI3K-Akt, cell adhesion molecules, MAPK signaling pathway, and ECM-receptor interaction. This finding further indicated that genetic alterations activate intracellular signaling pathways such as PI3K-Akt and MAPK pathways, which were implicated in thyroid cancer cell survival and proliferation [27,28]. To further reveal the pathological function of ITGA3 in papillary thyroid cancer, we performed GSEA to analyze the genome dataset of thyroid cancer samples with high and low expression groups of ITGA3. The results showed that ECM-receptor interaction and cell adhesion molecules were consistent pathways with KEGG analysis. Integrins are involved in mediating multiple intracellular signals via interaction with the ECM and participate in the attachment of cells to the ECM by the formation of cell adhesion complexes [29]. It has been reported that integrins played a regulatory role in angiogenesis, tumor growth, and invasion through interaction with the ECM in glioblastomas [30]. Accumulated evidence exhibited that integrins are expressed in stem cells and promote cell migration through ECM stimulation [31,32]. The inhibition of ITGA3 led to a decrease in the expression of cancer stem cell markers, indicating that the knockdown of ITGA3 impairs breast cancer cell stemness [33]. Similarly, thyroid cancer originates from stem cells [34], and hence it is reasonable to infer that ITGA3 overexpression might upregulate the expression of papillary thyroid cancer stem cell markers, and promote cancer progression via the ECM-receptor interaction pathway. Our study provided new insight into the development of papillary thyroid cancer.

We next explored the potential miRNAs targeting ITGA3 mRNA expression by using bioinformatics databases. Through Venn
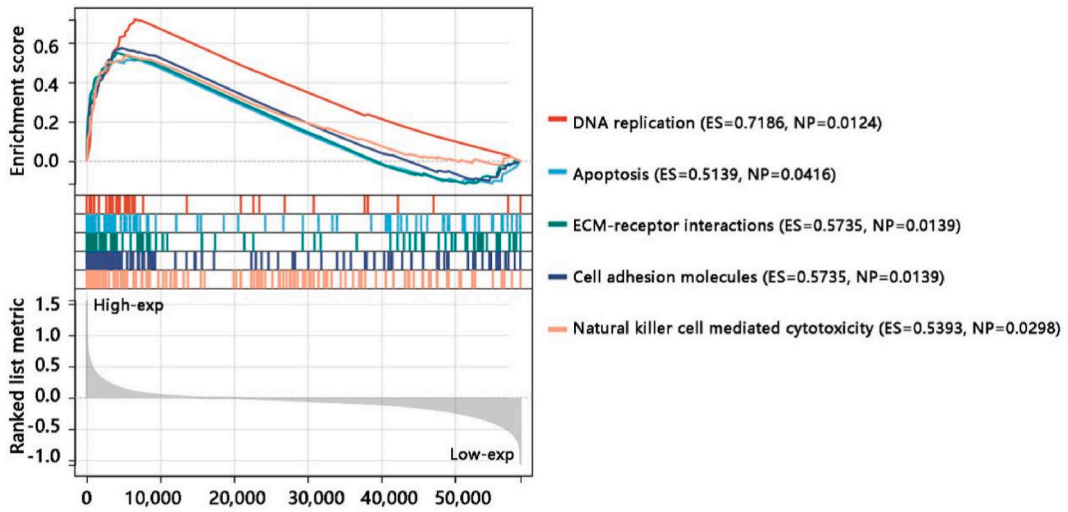
Fig. 7. Top 5 significant pathways enriched in high ITGA3 expression phenotypes. ES, enrichment score; NP, nominal *P*-value.
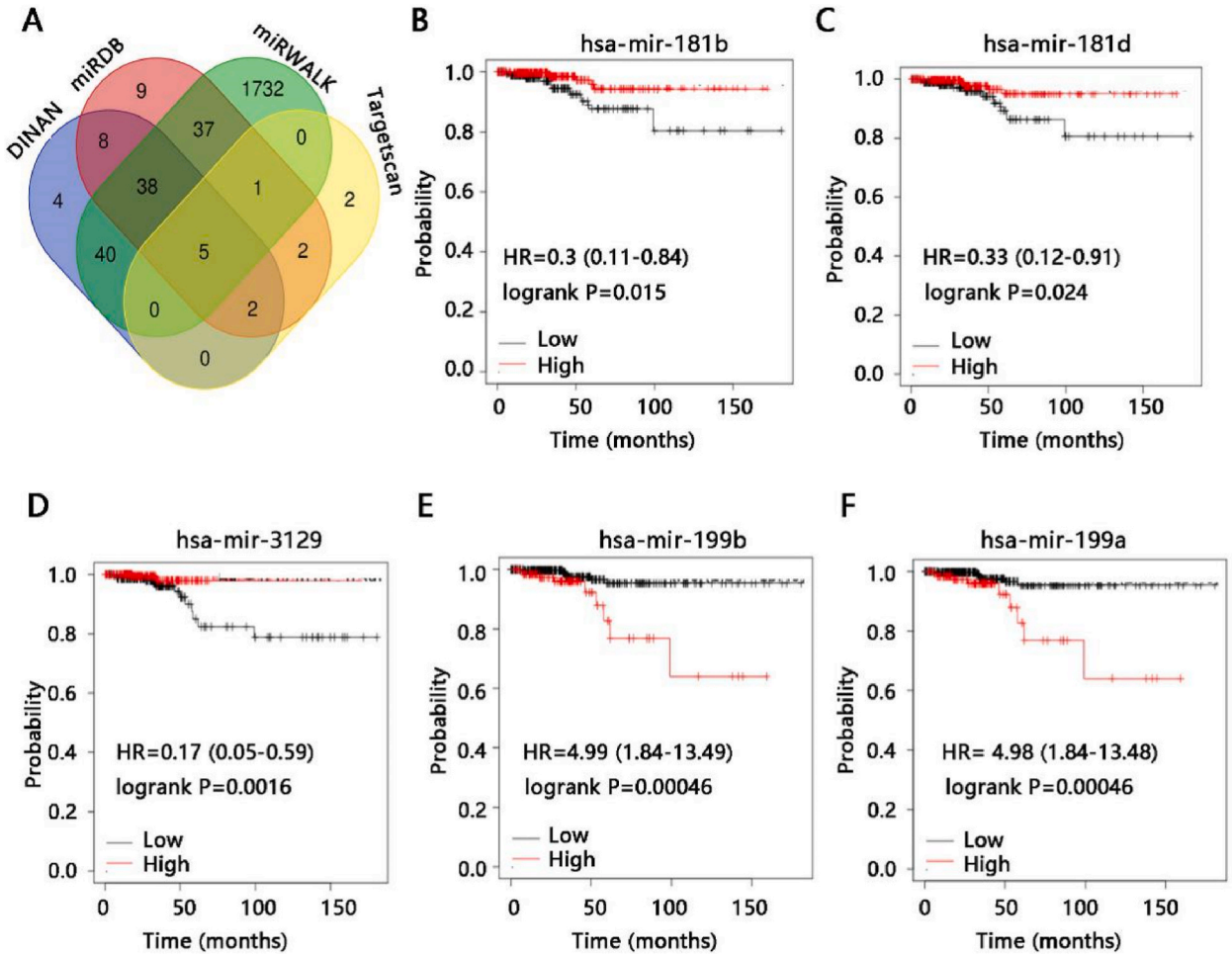

Fig. 8. Identification of upstream miRNAs of ITGA3. **(A)** The consistent miRNAs from 4 databases by Venn analysis. Prognostic value of **(B)** hsa-mir-181b, **(C)** hsa-mir-181d, **(D)** hsa-mir-3129, **(E)** hsa-mir-199b, and **(F)** hsa-mir-199a in papillary thyroid cancer.
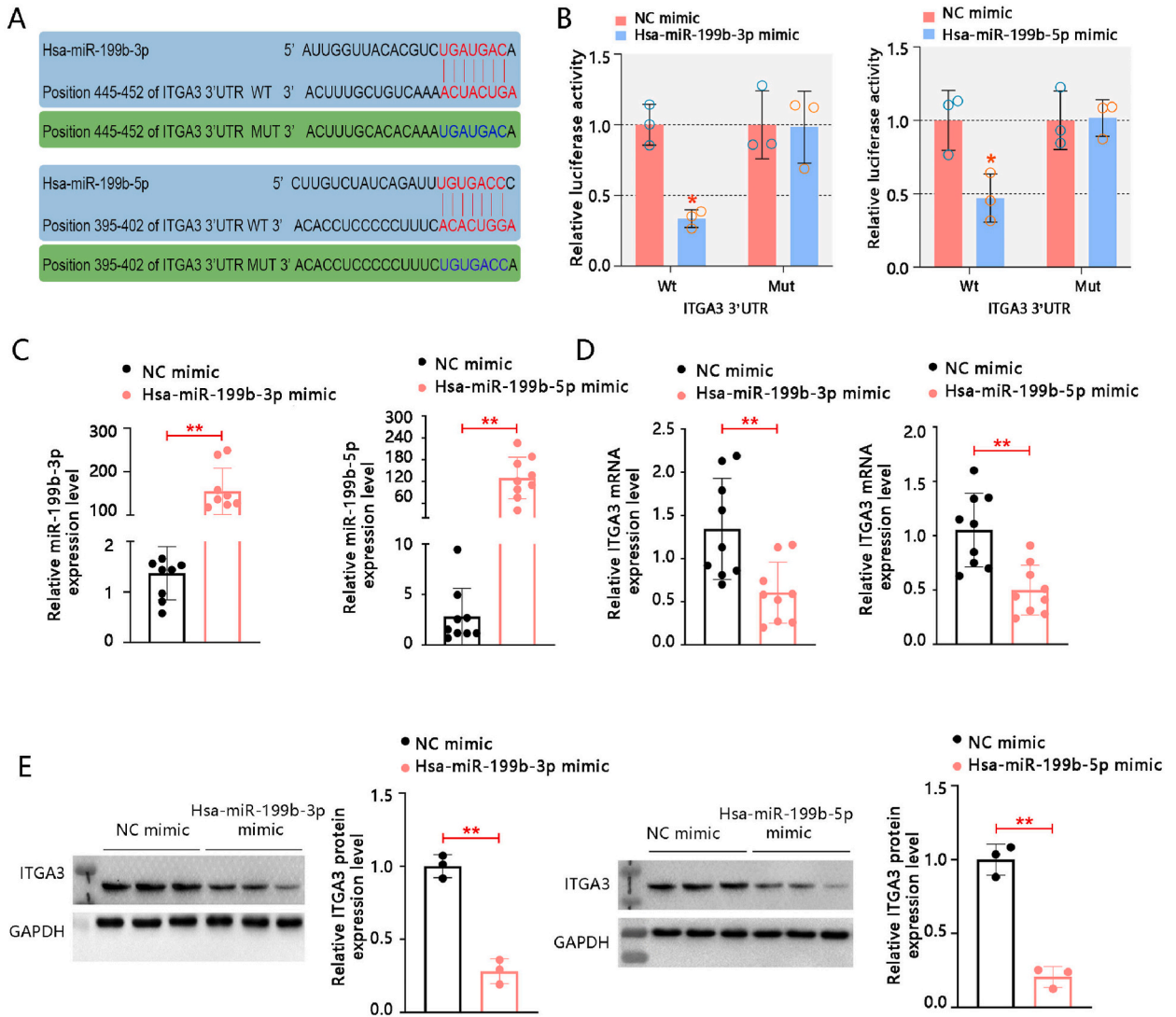
Fig. 9. The confirmation of hsa-miR-199b with ITGA3. **(A)** The binding site of has-miR-199b and ITGA3. **(B)** Dual-luciferase reporter assay was adopted to validate the binding site between miR-199b-3p/5p and ITGA3. **(C)** qRT-PCR confirmed the upregulated expression of has-miR-199b-3p/5p after transfection. **(D)** qRT-PCR exhibited the decreased expression of ITGA3 after miR-199b-3p/5p were overexpressed in the papillary thyroid cancer cell line MDA-T32. **(E)** Western blot assay confirmed the downregulation of ITGA3 after the overexpression of miR-199b-3p and miR-199b-5p. $*P < 0.05$, $**P < 0.01$.

analysis, hsa-mir-181b, hsa-mir-181d, hsa-mir-3129, hsa-mir-199b, and hsa-mir-199a were identified as consistent miRNAs from the four databases. As short endogenous non-coding molecules, miRNAs played predominant roles in the occurrence of many tumors [8, 35]. miRNAs modulate the expression of protein-coding/noncoding RNA by repressing translation or cleaving RNA transcripts in a sequence-dependent manner [36]. Gene expression regulated by miRNAs plays a significant role in many cellular processes such as cell differentiation, proliferation, and apoptosis [37]. It has been demonstrated that miR-3666 was related to thyroid cancer cell proliferation, and miR-146b-5p was involved in cell invasion and migration of different thyroid cancer cells [38,39]. This research revealed that hsa-mir-181b, hsa-mir-181d, hsa-mir-3129, hsa-mir-199b, and hsa-mir-199a were all associated with the prognosis of papillary thyroid cancer patients. Moreover, the correlation analysis exhibited that ITGA3 had a significant relationship with the consistent miRNAs except for hsa-mir-3129. Dual-luciferase reporter experiment confirmed the combination of has-miR-199b-3p/miR-199b-5p with ITGA3, and ITGA3 expression was reduced after has-miR-199b-3p/miR-199b-5p were overexpressed.

For strengths, we linked the expression of ITGA3 to the five consistent miRNAs, which provided a better understanding of the underlying mechanisms by which ITGA3 might affect papillary thyroid cancer cell proliferation, apoptosis, invasion, and migration. Besides, we combined the efficient bioinformatics approaches with experiments to assess the expression and prognosis of ITGA3 in papillary thyroid cancer and explored its potential underlying mechanism. However, the prognostic role of ITGA3 in papillary thyroid cancer should be verified in our own cohort if conditions were available in the future. Besides, the value of ITGA3 involved in papillary

thyroid cancer cell proliferation, apoptosis, invasion, and migration should be validated by *in vitro* experiments in the future.

In conclusion, our study showed that ITGA3 is highly expressed in papillary thyroid cancer and high expression of ITGA3 leads to worse RFS. In addition, ITGA3 had potential prognostic and predictive value for papillary thyroid cancer, which might be a robust biomarker for improving the diagnosis and prognosis of papillary thyroid cancer patients.

Data availability statement

Data will be made available on request.

Ethics statement

Approval by an ethics committee was not needed because the data in this study are from public databases.

Funding

Not applicable.

CRediT authorship contribution statement

Jun-jie Ma: Writing - original draft, Formal analysis, Data curation, Conceptualization. **Cheng Xiang:** Writing - original draft, Methodology, Data curation. **Heng-qing Zhu:** Writing - original draft, Formal analysis. **Bing-long Bai:** Writing - original draft, Methodology, Investigation. **Ping Wang:** Writing - original draft, Supervision, Data curation. **Guan-an Zhao:** Writing - review & editing, Methodology, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e23163.

References

[1] A. Jemal, et al., Cancer statistics, 2010, CA Cancer J Clin 60 (5) (2010) 277–300.
[2] M. Ning, et al., LncRNA AFAP-AS1 promotes anaplastic thyroid cancer progression by sponging miR-155-5p through ETS1/ERK pathway, Bioengineered 12 (1) (2021) 1543–1554.
[3] W. Chen, et al., Cancer statistics in China, 2015, CA Cancer J Clin 66 (2) (2016) 115–132.
[4] J.E. Maxwell, et al., Medical management of metastatic medullary thyroid cancer, Cancer 120 (21) (2014) 3287–3301.
[5] S.S. Islam, et al., Application of machine learning algorithms to predict the thyroid disease risk: an experimental comparative study, PeerJ Comput Sci 8 (2022) e898.
[6] R.L. Siegel, K.D. Miller, A. Jemal, Cancer statistics, 2020, CA Cancer J Clin 70 (1) (2020) 7–30.
[7] G. Zhu, et al., Clinical significance of the BRAFV600E mutation in PTC and its effect on radioiodine therapy, Endocr Connect 8 (6) (2019) 754–763.
[8] X. Shi, et al., Differential clinicopathological risk and prognosis of major papillary thyroid cancer variants, J. Clin. Endocrinol. Metab. 101 (1) (2016) 264–274.
[9] Y. Takada, X. Ye, S. Simon, The integrins, Genome Biol. 8 (5) (2007) 215.
[10] H. Kolarova, et al., Modulation of endothelial glycocalyx structure under inflammatory conditions, Mediat. Inflamm. 2014 (2014), 694312.
[11] M.Z. Gilcrease, Integrin signaling in epithelial cells, Cancer Lett. 247 (1) (2007) 1–25.
[12] V. Givant-Horwitz, B. Davidson, R. Reich, Laminin-induced signaling in tumor cells, Cancer Lett. 223 (1) (2005) 1–10.
[13] M. Barczyk, S. Carracedo, D. Gullberg, Integrins, Cell Tissue Res. 339 (1) (2010) 269–280.
[14] Y. Huang, et al., High expression of ITGA3 promotes proliferation and cell cycle progression and indicates poor prognosis in intrahepatic cholangiocarcinoma, BioMed Res. Int. 2018 (2018), 2352139.
[15] K. Koshizuka, et al., Regulation of ITGA3 by the anti-tumor miR-199 family inhibits cancer cell migration and invasion in head and neck cancer, Cancer Sci. 108 (8) (2017) 1681–1692.
[16] A. Kurozumi, et al., Tumor-suppressive microRNA-223 inhibits cancer cell migration and invasion by targeting ITGA3/ITGB1 signaling in prostate cancer, Cancer Sci. 107 (1) (2016) 84–94.
[17] C.L. Bi, et al., MicroRNA-520a-3p suppresses epithelial-mesenchymal transition, invasion, and migration of papillary thyroid carcinoma cells via the JAK1-mediated JAK/STAT signaling pathway, J. Cell. Physiol. 234 (4) (2019) 4054–4067.
[18] A. Lanczky, B. Gyorffy, Web-based survival analysis tool tailored for medical research (KMplot): development and implementation, J. Med. Internet Res. 23 (7) (2021), e27633.

[19] I.J. Nixon, A.R. Shaha, Implications of extra nodal extension in differentiated thyroid cancer, J. Surg. Oncol. 116 (4) (2017) 459–460.

[20] N. Veronese, et al., Prognostic impact of extra-nodal extension in thyroid cancer: a meta-analysis, J. Surg. Oncol. 112 (8) (2015) 828–833.

[21] H.S. Kazaure, S.A. Roman, J.A. Sosa, The impact of age on thyroid cancer staging, Curr. Opin. Endocrinol. Diabetes Obes. 25 (5) (2018) 330–334.

[22] A. Faggiano, et al., Age-dependent variation of follicular size and expression of iodine transporters in human thyroid tissue, J. Nucl. Med. 45 (2) (2004) 232–237.

[23] S. Cartwright, A. Fingeret, Contemporary evaluation and management of tall cell variant of papillary thyroid carcinoma, Curr. Opin. Endocrinol. Diabetes Obes. 27 (5) (2020) 351–357.

[24] A. Bommarito, et al., BRAFV600E mutation, TIMP-1 upregulation, and NF-kappaB activation: closing the loop on the papillary thyroid cancer trilogy, Endocr. Relat. Cancer 18 (6) (2011) 669–685.

[25] D. Liu, et al., BRAF V600E maintains proliferation, transformation, and tumorigenicity of BRAF-mutant papillary thyroid cancer cells, J. Clin. Endocrinol. Metab. 92 (6) (2007) 2264–2271.

[26] F. Fang, et al., Breast cancer methylomes establish an epigenomic foundation for metastasis, Sci. Transl. Med. 3 (75) (2011) 75ra25.

[27] M. Santoro, R.M. Melillo, A. Fusco, RET/PTC activation in papillary thyroid carcinoma: European Journal of Endocrinology Prize Lecture, Eur. J. Endocrinol. 155 (5) (2006) 645–653.

[28] E. Miyagi, et al., Chronic expression of RET/PTC 3 enhances basal and insulin-stimulated PI3 kinase/AKT signaling and increases IRS-2 expression in FRTL-5 thyroid cells, Mol. Carcinog. 41 (2) (2004) 98–107.

[29] M. Nakada, et al., Integrin alpha3 is overexpressed in glioma stem-like cells and promotes invasion, Br. J. Cancer 108 (12) (2013) 2516–2524.

[30] M. Nakada, et al., The phosphorylation of EphB2 receptor regulates migration and invasion of human glioma cells, Cancer Res. 64 (9) (2004) 3179–3185.

[31] J.S. Desgrosellier, D.A. Cheresh, Integrins in cancer: biological implications and therapeutic opportunities, Nat. Rev. Cancer 10 (1) (2010) 9–22.

[32] S.J. Ellis, G. Tanentzapf, Integrin-mediated adhesion and stem-cell-niche interactions, Cell Tissue Res. 339 (1) (2010) 121–130.

[33] S. Ardalan Khales, et al., Linkage between EMT and stemness state through molecular association between TWIST1 and NY-ESO1 in esophageal squamous cell carcinoma, Biochimie 163 (2019) 84–93.

[34] M. Todaro, et al., Tumorigenic and metastatic activity of human thyroid cancer stem cells, Cancer Res. 70 (21) (2010) 8874–8885.

[35] J. Xiang, et al., miR-584 suppresses invasion and cell migration of thyroid carcinoma by regulating the target oncogene ROCK1, Oncol. Res. Treat. 38 (9) (2015) 436–440.

[36] D.P. Bartel, MicroRNAs: target recognition and regulatory functions, Cell 136 (2) (2009) 215–233.

[37] L.A. Macfarlane, P.R. Murphy, MicroRNA: biogenesis, function and role in cancer, Curr. Genom. 11 (7) (2010) 537–561.

[38] G. Wang, C. Cai, L. Chen, MicroRNA-3666 regulates thyroid carcinoma cell proliferation via MET, Cell. Physiol. Biochem. 38 (3) (2016) 1030–1039.

[39] C.R. Lima, et al., MiRNA-146b-5p upregulates migration and invasion of different Papillary Thyroid Carcinoma cells, BMC Cancer 16 (2016) 108.