Increased Expression of Integrin Subunit $\alpha 3$ is Correlated with Worse Recurrence-Free Survival in Thyroid Cancer

Jun-jie Ma

The Second Affiliated Hospital of Zhejiang University School of Medicine

Cheng Xiang

The Second Affiliated Hospital of Zhejiang University School of Medicine

Heng-qing Zhu

The Second Affiliated Hospital of Zhejiang University School of Medicine

Bing-long Bai

The Second Affiliated Hospital of Zhejiang University School of Medicine

Ping Wang (✉ p.wang@zju.edu.cn)

The Second Affiliated Hospital of Zhejiang University School of Medicine

Research Article

Keywords: Thyroid cancer (THCA), Integrin subunit $\alpha 3$ (ITGA3), Prognosis, Biomarker

Posted Date: December 28th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1190805/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Objective: To investigate the potential effect of integrin subunit $\alpha 3$ (ITGA3) on thyroid cancer (THCA).

Materials and Methods: Based on bioinformatics databases, the expression levels of ITGA3 were firstly analyzed, followed by evaluating the prognostic significance of ITGA3 in THCA patients. Cox regression analysis predicted the independent prognostic factors for THCA. Then, cBioportal and GSCA databases were applied to evaluate genetic alterations of ITGA3. Functional enrichment analysis was conducted using the R package. Finally, the upstream microRNAs of ITGA3 were determined.

Results: ITGA3 gene and protein levels were higher in the THCA group than normal group (all $P < 0.05$). And ITGA3 mRNA expression was significantly related to cancer stage and histological subtype (all $P < 0.01$). Moreover, high expression of ITGA3 was associated with poor recurrence-free survival (RFS) of THCA patients in all subgroups (all $P < 0.01$). Cox regression analysis presented that ITGA3 overexpression was an independent prognostic factor for worse RFS in THCA patients. Besides, significant relations were observed between ITGA3 and genetic alterations ($FDR < 0.01$). Functional enrichment analysis indicated ECM-receptor interaction, and cell adhesion molecules were the shared regulatory pathways. We also found that ITGA3 might be the target gene of hsa-miR-3129, hsa-miR-181d, hsa-miR-181b, hsa-miR-199a, and hsa-miR-199b, which were all correlated with THCA patient prognosis.

Conclusions: ITGA3 could be a reliable biomarker and promising therapeutic target for improving the diagnosis and clinical outcomes of THCA patients.

Introduction

Thyroid cancer (THCA) is the most common endocrine malignancy, which was secondary to cancers of breast, lung, rectal, and cervical in 2017 among female malignancies [1]. In China, there were approximately 90,000 THCA cases in 2015 [2]. It is estimated that the number of new THCA cases reached 12.9 per 100,000 populations annually in 2015 in the United States [3]. According to the statistics published by the American Cancer Society, about 12,150 and 32,130 new THCA cases occurred in males and females, respectively in 2021. Importantly, THCA would be responsible for 2,200 deaths including 1,150 females and 1,050 males [<https://www.cancer.org/cancer/thyroid-cancer/about/key-statistics.html>]. Currently, fine-needle aspiration biopsy of thyroid nodules is a paramount method for the diagnosis of THCA due to its high accuracy in distinguishing benign from malignant lesions. However, clinicians are faced with a big challenge in distinguishing malignant follicular lesions from benign lesions on biopsy [4-6]. In addition, despite the majority of THCA patients having a good prognosis, recurrences are observed in nearly 30% of the patients [7]. Therefore, it is urgent to develop novel biomarkers with good sensitivity and specificity to improve the diagnosis and prognosis of THCA patients.

Integrins are cell surface receptors for extracellular matrix (ECM) proteins, which are widely expressed in tissues and organs in the human body [8]. They represent a crucial role in the proliferation, migration,

invasion, and metastasis of the cancer cells [9, 10]. As a member of the integrin family, integrin subunit $\alpha 3$ (ITGA3) joins a $\beta 1$ subunit to form an intact integrin and interacts with ECM proteins. Previous studies have demonstrated abnormal expression of ITGA3 in various cancers. Huang et al. showed that ITGA3 was highly expressed in intrahepatic cholangiocarcinoma, promoting cell proliferation and cell cycle progression [11]. The elevated levels of ITGA3 in head and neck cancer led to an unfavorable prognosis for the patients [12]. In addition, ITGA3 was directly regulated by miR-223, and silencing of ITGA3 significantly inhibited prostate cancer cell migration and invasion [13]. However, there have been few reports regarding the role of ITGA3 in THCA.

In this study, we aimed to explore the relationship between ITGA3 expression and the prognosis of THCA patients from the following aspects: the expression levels of ITGA3 were firstly analyzed through various bioinformatics databases. Then, the prognostic significance of ITGA3 mRNA expression in THCA was evaluated through Kaplan-Meier plotter and Cox regression analyses. By identifying the differentially expressed genes (DEGs), the gene ontology (GO) and kyoto encyclopedia of genes and genomes (KEGG) pathway analyses were performed, gene set enrichment analysis (GSEA) was conducted, and the upstream microRNAs (miRNAs) of ITGA3 were identified to reveal the underlying molecular mechanisms of abnormal ITGA3 in THCA.

Materials And Methods

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/#/>). The GEPIA database (<http://gepia.cancer-pku.cn/>) was used to analyze the mRNA expression of ITGA3 in THCA and normal samples by setting the parameters of $|\log_2\text{-fold change (FC)}|$ cutoff =1 and P-value =0.01. Then, RNA-seq-HTseq-FPKM data for THCA 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 THCA and paired normal tissues. Moreover, the mRNA expression of ITGA3 in THCA 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.

Following this, the protein expression levels of ITGA3 in THCA and normal thyroid tissues were evaluated by the Human Protein Atlas (HPA) database (<https://www.proteinatlas.org/>) in the "PATHOLOGY" column. The levels of expression were classified into not detected, low, medium, and high through the score system, including the intensity of staining (negative, weak, moderate, or strong) and the proportion of stained cells (<25%, 25-75%, or >75%). In this database, we also explored the subcellular distribution pattern of ITGA3 proteins in human cells. Three cell lines, A-431, U-2 OS, and U-251 MG, were included in the analysis of protein subcellular localization by immunofluorescence. The HPA database maps all the human proteins in cells, tissues, and organs using an integration of various omics technologies.

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) on THCA patients by splitting patients with the best cutoff [14]. Then, the clinical information and related RNA-seq data were retrieved from cBioportal for Cancer Genomics (<https://www.cbioportal.org/>) by choosing “Thyroid carcinoma (TCGA, Firehose Legacy)” and querying “ITGA3”. Patients with complete survival information and ITGA3 expression data were enrolled in the study. We used “survfit” in R package to analyze the association of ITGA3 expression with clinical outcome of THCA patients according to age (<55/ ≥55), 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. Further, univariate and multivariate Cox regression analyses were performed to predict the independent prognostic factors for RFS in THCA patients. Female was set as a reference level for gender, stage 1+2 for stage, and Asian for race.

Mutation, Copy Number Variation (CNV), and methylation analysis of ITGA3

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

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 THCA. An absolute $\log_2FC > 1$ and $P\text{-value} < 0.05$ were used as the filter criterion for significantly DEGs. The significantly enriched GO terms and the KEGG pathways of these DEGs were investigated using the clusterProfiler in R package. $P\text{-value} < 0.05$ 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 permuted 1000 times and the expression level of ITGA3 was used as a phenotypic label. A nominal $p\text{-value} < 0.05$ and an FDR $q\text{-value} < 0.25$ were considered to be statistically significant.

Identification of upstream miRNAs

To further understand the underlying mechanism of THCA, 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 THCA patients was evaluated via the Kaplan-Meier plotter website. Finally, the interactions

between ITGA3 and the predicted miRNAs were explored by the starBase v 3.0 (<https://starbase.sysu.edu.cn/index.php>).

Statistical analysis

All statistical analyses were performed by SPSS 23.0 (SPSS, Inc., Chicago, IL, USA) and R software. Paired t-test was used to assess the differential expression between THCA and paired normal tissues. 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. While the Pearson correlation test was utilized to evaluate the relationship between ITGA3 expression and miRNAs in starBase. $P < 0.05$ was considered statistically significant.

Results

Expression analysis of ITGA3

Firstly, we analyzed the ITGA3 mRNA expression in various cancers through the GSCA database. As shown in **Figure 1A**, ITGA3 was highly expressed in 9 types of cancer including THCA (all $P < 0.05$). Quantitative analysis confirmed ITGA3 overexpression in THCA compared with normal thyroid tissue ($P < 0.05$) (**Figure 1B**). Besides, the THCA group had a significantly higher ITGA3 mRNA expression than that in the paired normal group ($P < 0.001$) (**Figure 1C**). Further, the mRNA expression of ITGA3 in THCA based on clinical characteristics including age, gender, cancer stage, and histological subtype were analyzed through the UALCAN database. There was no significant difference in age and gender (**Figure 2A-2B**), but cancer stage and histological subtype were significantly associated with ITGA3 expression (all $P < 0.01$) (**Figure 2C-2D**).

Next, the protein expression of ITGA3 in THCA and normal tissues were explored via the HPA database. **Figure 1D** showed that the protein staining of ITGA3 was high and the intensity was strong in THCA tissue; however, low staining and weak intensity were observed in normal tissue. **Figure 1E** presented the ITGA3 protein localization and immunofluorescence result, showing that the ITGA3 protein was mainly located at the plasma membrane in human tumor cells. Taken together, the above findings indicated that ITGA3 was highly expressed in THCA and might be related to cancer development.

Survival analysis of ITGA3

To investigate the effect of ITGA3 on OS and RFS in THCA patients, the survival analysis was carried out using a Kaplan-Meier plotter. As exhibited in **Figure 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 ($P < 0.01$) (**Figure 3B**). Further stratified analysis showed that high ITGA3 expression correlated with poor RFS in all the subgroups (**Table 1**).

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 2**).

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 in THCA patients (**Table 2**). These results revealed that ITGA3 played a vital role in the prognosis of THCA.

Table 1. The correlation between ITGA3 mRNA expression and recurrence-free survival in 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	255	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	101	4.88 (1.84-12.95)	4.4e-4

Abbreviations: CI, confidence interval.

Table 2. Cox regression analysis of factors associated with recurrence-free survival in 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.

Mutation, CNV, and methylation analyses of ITGA3

Due to the independent prognostic value of ITGA3 for RFS in THCA 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 D790N, and R874L (**Figure 4A**). Besides, the mRNA expression of ITGA3 showed a significantly negative correlation with CNV (Cor. =-0.16, FDR =3.1e-03) (**Figure 4B**). Similarly, the mRNA expression was negatively related to DNA methylation (Cor. =-0.42, FDR <0.001) (**Figure 4C**). Therefore, the mutations, CNV, and DNA methylation might lead to the aberrant upregulation of ITGA3 in THCA.

Functional enrichment analysis

To elucidate the pathological role of ITGA3 in THCA, the GO and KEGG analysis of the DEGs between the low and high ITGA3 expression groups were performed using 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 (**Figure 5A**) and the heat map (**Figure 5B**). 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 (**Figure 5C**). As for the KEGG pathway, they were mainly enriched in PI3K-Akt, cell adhesion molecules, MAPK signaling pathway, and ECM-receptor interaction (**Figure 5D**).

Then, GSEA was conducted to gain further insight into the biological pathways involved in THCA. Among the top 5 pathways in high ITGA3 expression phenotype, ECM-receptor interaction, and cell adhesion molecules were the consistent pathways with KEGG analysis (**Figure 6**). Thus, high expression of ITGA3 contributed to the poor prognosis of THCA patients, which might be related to the activation of these pathways, especially ECM-receptor interaction, and cell adhesion molecules.

Upstream miRNA identification of ITGA3

To further reveal the underlying mechanism of THCA, 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 (**Figure 7A**). 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 of THCA patients (all P <0.05) (**Figure 7B-7D**). Nevertheless, the patients with high hsa-mir-199b and hsa-mir-199a had a shorter survival time (all P <0.001) (**Figure 7E-7F**). The results revealed that hsa-miR-3129, hsa-miR-181d, hsa-miR-181b, hsa-miR-199a, and hsa-miR-199b might be essential in cancer progression by different regulations. More importantly, hsa-mir-181b, hsa-mir-181d, hsa-miR-199a, and hsa-miR-199b were positively correlated with ITGA3 expression with statistical significance (all P <0.001) (**Figure 8**).

Discussion

THCA accounts for more than 95% of endocrine cancer, and most are well-differentiated arising from follicular epithelial cells [15]. Although THCA patients have better clinical outcomes, a number of patients still develop local recurrence with or without cervical lymph node metastasis or distant metastases [16]. With the development of molecular biology technology, researches on THCA have also been progressed [17]. Previous studies have demonstrated that high expression of COX-2 was related to the pathological type of THCA, and its overexpression contributed to poor prognosis [18, 19]. Fu et al. confirmed that TFAP2B overexpression accelerated tumor growth and progression of THCA via the COX-2 signaling pathway [16]. Besides, PTPN2 upregulated by inflammatory response or oxidative stress promoted the THCA development [20]. However, little is known about the expression of ITGA3 and prognostic value in THCA.

In this study, we found that ITGA3 was highly expressed at both gene and protein levels in the THCA group compared with the normal group. 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. Cox regression analysis exhibited that the upregulated expression of ITGA3 served as an independent prognostic factor for worse RFS of THCA patients. Of note, ITGA3 overexpression predicted poor clinical outcomes in all subgroups. Thus, ITGA3 might be a novel and robust prognostic biomarker for THCA.

Tumor-initiating genetic events have become a primary focus in THCA initiation in the recent decade [21, 22]. By analyzing the TCGA data, we found that mutations, CNV, and methylation might result in the aberrant elevation of ITGA3 expression in THCA. In addition, GO and KEGG analysis of DEGs based on ITGA3 expression levels showed that ITGA3 regulated the development of THCA 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 THCA cell survival and proliferation [23, 24]. To further reveal the pathological function of ITGA3 in THCA, we performed GSEA to analyze the genome dataset of THCA samples with high and low expression groups of ITGA3. The results showed that ECM-receptor interaction and cell adhesion molecules were the 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 [25]. It has been reported that integrins played a regulatory role in angiogenesis, tumor growth, and invasion through interaction with the ECM in glioblastomas [26]. Accumulated evidence exhibited that integrins are expressed in stem cells and promote cell migration through ECM stimulation [27, 28]. 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 [29]. Similarly, THCA originates from stem cells [30], and hence it is reasonable to infer that ITGA3 overexpression might upregulate the expression of THCA stem cell markers, and promote cancer progression via the ECM-receptor interaction pathway. Our study provided new insight into the development of THCA.

We next explored the potential miRNAs targeting ITGA3 mRNA expression by using DINAN, miRDB, miRWALK, and Targetscan databases. Through Venn analysis, has-mir-181b, has-mir-181d, has-mir-3129, has-mir-199b, and has-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 [7, 31]. It has been demonstrated that miR-3666 was related to THCA cell proliferation, and miR-146b-5p was involved in cell invasion and migration of different THCA cells [32, 33]. This research revealed that has-mir-181b, has-mir-181d, has-mir-3129, has-mir-199b, and has-mir-199a were all associated with the prognosis of THCA patients. Moreover, the correlation analysis exhibited that ITGA3 had a significant relationship with the consistent miRNAs except for has-mir-3129. For the first time, we linked the expression of ITGA3 to the five consistent miRNAs, which provided a better understanding of the underlying mechanisms by which ITGA3 affected THCA progression. However, *in vitro* and *in vivo* experiments are required to validate these results if conditions are available in the future.

In conclusion, our study showed that ITGA3 was highly expressed in THCA and high expression of ITGA3 led to worse RFS of THCA patients. Besides, ITGA3 might affect the progression of THCA by regulating the ECM-receptor interaction pathway. ITGA3 might serve as a vital predictor and potential target for THCA treatment.

Abbreviations

GSCA, Gene Set Cancer Analysis; FDR, false discovery rate; ECM, extracellular matrix.

Declarations

Availability of data and materials: The dataset used and/or analyzed during the current study is available from the corresponding author on reasonable request.

Competing interests: The authors have no conflicts of interest to declare.

Author contributions:

(I) Conception and design: Jun-jie Ma and Cheng Xiang

(II) Collection and assembly of data: Heng-qing Zhu

(III) Data analysis and interpretation: Bing-long Bai and Ping Wang

(IV) Manuscript writing: All authors

(V) Final approval of manuscript: All authors

Consent for publication: Not applicable.

Ethics approval and consent to participate: Not applicable.

Funding: No funding was received for conducting this study.

Acknowledgement: Not applicable.

References

1. Jemal A, Siegel R, Xu J, Ward E. 2010**Cancer statistics, 2010.***CA Cancer J Clin*, **60**:277-300. doi:10.3322/caac.20073.
2. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, et al. 2016**Cancer statistics in China, 2015.***CA Cancer J Clin*, **66**:115-132. doi:10.3322/caac.21338.
3. Maxwell JE, Sherman SK, O'Dorisio TM, Howe JR. 2014**Medical management of metastatic medullary thyroid cancer.***Cancer*, **120**:3287-3301. doi:10.1002/cncr.28858.
4. Lansford CD, Teknos TN. 2006**Evaluation of the thyroid nodule.***Cancer Control*, **13**:89-98. doi:10.1177/107327480601300202.
5. American Thyroid Association Guidelines Taskforce on Thyroid N, Differentiated Thyroid C, Cooper DS, Doherty GM, Haugen BR, et al. 2009**Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer.***Thyroid*, **19**:1167-1214. doi:10.1089/thy.2009.0110.
6. Castro MR, Gharib H. 2005**Continuing controversies in the management of thyroid nodules.***Ann Intern Med*, **142**:926-931. doi:10.7326/0003-4819-142-11-200506070-00011.
7. Shi X, Liu R, Basolo F, Giannini R, Shen X, et al. 2016**Differential Clinicopathological Risk and Prognosis of Major Papillary Thyroid Cancer Variants.***J Clin Endocrinol Metab*, **101**:264-274. doi:10.1210/jc.2015-2917.
8. Takada Y, Ye X, Simon S. 2007**The integrins.***Genome Biol*, **8**:215. doi:10.1186/gb-2007-8-5-215.
9. Gilcrease MZ. 2007**Integrin signaling in epithelial cells.***Cancer Lett*, **247**:1-25. doi:10.1016/j.canlet.2006.03.031.
10. Givant-Horwitz V, Davidson B, Reich R. 2005**Laminin-induced signaling in tumor cells.***Cancer Lett*, **223**:1-10. doi:10.1016/j.canlet.2004.08.030.
11. Huang Y, Kong Y, Zhang L, He T, Zhou X, et al. 2018**High Expression of ITGA3 Promotes Proliferation and Cell Cycle Progression and Indicates Poor Prognosis in Intrahepatic Cholangiocarcinoma.***Biomed Res Int*, **2018**:2352139. doi:10.1155/2018/2352139.
12. Koshizuka K, Hanazawa T, Kikkawa N, Arai T, Okato A, et al. 2017**Regulation of ITGA3 by the anti-tumor miR-199 family inhibits cancer cell migration and invasion in head and neck cancer.***Cancer Sci*, **108**:1681-1692. doi:10.1111/cas.13298.
13. Kurozumi A, Goto Y, Matsushita R, Fukumoto I, Kato M, et al. 2016**Tumor-suppressive microRNA-223 inhibits cancer cell migration and invasion by targeting ITGA3/ITGB1 signaling in prostate cancer.***Cancer Sci*, **107**:84-94. doi:10.1111/cas.12842.

14. Lanczky A, Gyorffy B. 2021 **Web-Based Survival Analysis Tool Tailored for Medical Research (KMplot): Development and Implementation.***J Med Internet Res*, **23**:e27633. doi:10.2196/27633.
15. Marques P, de Vries F, Dekkers OM, Korbonits M, Biermasz NR, et al. 2021 **Serum Inflammation-based Scores in Endocrine Tumors.***J Clin Endocrinol Metab*, **106**:e3796-e3819. doi:10.1210/clinem/dgab238.
16. Fu X, Zhang H, Chen Z, Yang Z, Shi D, et al. 2019 **TFAP2B overexpression contributes to tumor growth and progression of thyroid cancer through the COX-2 signaling pathway.***Cell Death Dis*, **10**:397. doi:10.1038/s41419-019-1600-7.
17. Mohammadi M, Hedayati M. 2017 **A Brief Review on The Molecular Basis of Medullary Thyroid Carcinoma.***Cell J*, **18**:485-492. doi:10.22074/cellj.2016.4715.
18. Ji B, Liu Y, Zhang P, Wang Y, Wang G. 2012 **COX-2 expression and tumor angiogenesis in thyroid carcinoma patients among northeast Chinese population-result of a single-center study.***Int J Med Sci*, **9**:237-242. doi:10.7150/ijms.4173.
19. Siironen P, Ristimäki A, Narko K, Nordling S, Louhimo J, et al. 2006 **VEGF-C and COX-2 expression in papillary thyroid cancer.***Endocr Relat Cancer*, **13**:465-473. doi:10.1677/erc.1.01114.
20. Zhang Z, Xu T, Qin W, Huang B, Chen W, et al. 2020 **Upregulated PTPN2 induced by inflammatory response or oxidative stress stimulates the progression of thyroid cancer.***Biochem Biophys Res Commun*, **522**:21-25. doi:10.1016/j.bbrc.2019.11.047.
21. Bommarito A, Richiusa P, Carissimi E, Pizzolanti G, Rodolico V, et al. 2011 **BRAFV600E mutation, TIMP-1 upregulation, and NF-kappaB activation: closing the loop on the papillary thyroid cancer trilogy.***Endocr Relat Cancer*, **18**:669-685. doi:10.1530/ERC-11-0076.
22. Liu D, Liu Z, Condouris S, Xing M. 2007 **BRAF V600E maintains proliferation, transformation, and tumorigenicity of BRAF-mutant papillary thyroid cancer cells.***J Clin Endocrinol Metab*, **92**:2264-2271. doi:10.1210/jc.2006-1613.
23. Santoro M, Melillo RM, Fusco A. 2006 **RET/PTC activation in papillary thyroid carcinoma: European Journal of Endocrinology Prize Lecture.***Eur J Endocrinol*, **155**:645-653. doi:10.1530/eje.1.02289.
24. Miyagi E, Braga-Basaria M, Hardy E, Vasko V, Burman KD, et al. 2004 **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**:98-107. doi:10.1002/mc.20042.
25. Nakada M, Nambu E, Furuyama N, Yoshida Y, Takino T, et al. 2013 **Integrin alpha3 is overexpressed in glioma stem-like cells and promotes invasion.***Br J Cancer*, **108**:2516-2524. doi:10.1038/bjc.2013.218.
26. Nakada M, Niska JA, Miyamori H, McDonough WS, Wu J, et al. 2004 **The phosphorylation of EphB2 receptor regulates migration and invasion of human glioma cells.***Cancer Res*, **64**:3179-3185. doi:10.1158/0008-5472.can-03-3667.
27. Desgrosellier JS, Cheresh DA. 2010 **Integrins in cancer: biological implications and therapeutic opportunities.***Nat Rev Cancer*, **10**:9-22. doi:10.1038/nrc2748.

28. Ellis SJ, Tanentzapf G. 2010**Integrin-mediated adhesion and stem-cell-niche interactions.***Cell Tissue Res*, **339**:121-130. doi:10.1007/s00441-009-0828-4.
29. Ardalan Kholes S, Abbaszadegan MR, Majd A, Forghanifard MM. 2019**Linkage between EMT and stemness state through molecular association between TWIST1 and NY-ESO1 in esophageal squamous cell carcinoma.***Biochimie*, **163**:84-93. doi:10.1016/j.biochi.2019.05.016.
30. Todaro M, Iovino F, Eterno V, Cammareri P, Gambarà G, et al. 2010**Tumorigenic and metastatic activity of human thyroid cancer stem cells.***Cancer Res*, **70**:8874-8885. doi:10.1158/0008-5472.CAN-10-1994.
31. Xiang J, Wu Y, Li DS, Wang ZY, Shen Q, et al. 2015**miR-584 Suppresses Invasion and Cell Migration of Thyroid Carcinoma by Regulating the Target Oncogene ROCK1.***Oncol Res Treat*, **38**:436-440. doi:10.1159/000438967.
32. Wang G, Cai C, Chen L. 2016**MicroRNA-3666 Regulates Thyroid Carcinoma Cell Proliferation via MET.***Cell Physiol Biochem*, **38**:1030-1039. doi:10.1159/000443054.
33. Lima CR, Geraldo MV, Fuziwara CS, Kimura ET, Santos MF. 2016**MiRNA-146b-5p upregulates migration and invasion of different Papillary Thyroid Carcinoma cells.***BMC Cancer*, **16**:108. doi:10.1186/s12885-016-2146-z.

Figures

Figure 1

The gene and protein expression of ITGA3. **(A)** ITGA3 mRNA expression in various cancers. **(B)** The differential mRNA expression of ITGA3 in thyroid cancer and normal tissues. * $P < 0.05$. **(C)** The differential mRNA expression of ITGA3 in thyroid cancer and paired normal tissues. **(D)** The ITGA3 protein expression in thyroid cancer and normal tissues. **(E)** ITGA3 subcellular localization in tumor cells. Green: antibody; blue: nucleus; red: microtubules; yellow: endoplasmic reticulum.

Figure 2

The mRNA expression of ITGA3 in THCA based on clinical characteristics. **(A)** Age. **(B)** Gender. **(C)** Cancer stage. **(D)** Histological subtype. **P < 0.01, ***P < 0.001.

Figure 3

The effect of ITGA3 on overall survival and recurrence-free survival in thyroid cancer. **(A)** OS. **(B)** RFS. OS, overall survival; RFS, recurrence-free survival; HR, hazard ratio.

Figure 4

Mutation, copy number variation, and methylation of ITGA3 in 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.

Figure 5

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.

Figure 6

Top 5 significant pathways enriched in high ITGA3 expression phenotypes. ES, enrichment score; NP, nominal P-value.

Figure 7

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 thyroid cancer.

Figure 8

The significant relationship between ITGA3 and hsa-mir-181b, hsa-mir-181d, hsa-mir-199a, and hsa-mir-199a.