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lncRNA ACVR2B-AS1 modulates thyroid cancer progression by regulating miR-195-5p

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Abstract

Background lncRNAs are key regulators in thyroid cancer (TC). While lncRNA ACVR2B-AS1 has been proposed as a potential TC biomarker, its role remains underexplored. This study aims to clarify its clinical significance in TC and investigate its molecular mechanism.

Materials and methods qRT-PCR was used to assess the expression of ACVR2B-AS1 in TC tissues and cell lines. Kaplan–Meier survival curves and Cox regression were utilized to assess the prognostic value of ACVR2B-AS1 expression. The interaction between ACVR2B-AS1 and miR-195-5p, as well as their effects on cell viability, migration, and invasion, were evaluated using dual-luciferase reporter assays, CCK-8 assays, and Transwell assays.

Results ACVR2B-AS1 was significantly upregulated in TC tissues and cell lines, and its expression correlated with TNM stage and lymph node metastasis. Elevated ACVR2B-AS1 levels were associated with poor survival outcomes, and it was identified as an independent risk factor for TC progression. A direct regulatory relationship was established between ACVR2B-AS1 and miR-195-5p, with ACVR2B-AS1 negatively regulating miR-195-5p, thereby promoting TC cell proliferation, migration, and invasion. FGF2 was predicted and validated as a target gene of miR-195-5p.

Conclusion lncRNA ACVR2B-AS1 shows potential as a prognostic marker in TC and may regulate tumor progression through the miR-195-5p/FGF2 axis, offering new insights for TC diagnosis and treatment.

Keywords lncRNA ACVR2B-AS1 · Thyroid cancer · miR-195-5p · Clinical significance · Molecular mechanisms

Tianshi Qin and Chengqiang Lei should be considered joint first author.

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1 Introduction

In recent years, the incidence of thyroid cancer (TC) diagnoses has been steadily increasing, making it one of the most prevalent endocrine malignancies [1]. The etiology of TC is complex, with potential links to prolonged chemical exposure, poor diets, and unfavorable environmental factors [2]. Current treatments include targeted therapies, surgical, chemotherapy, radiotherapy, thyroid hormone suppression, and radioactive iodine [3]. However, many patients still face recurrence, metastasis, and treatment resistance [4]. Thus, understanding the molecular mechanisms underlying TC and identifying novel prognostic markers and therapeutic targets are essential for improving patient outcomes and quality of life [5].

Long non-coding RNAs (lncRNAs), which do not code for proteins, play crucial roles in various biological processes including regulating gene expression, modifying chromatin structure, RNA splicing, and translation [6, 7]. lncRNAs have gained significant attention in cancer research due to their involvement in the progression of various malignancies [7]. ACVR2B-AS1, located at 3p22.2, is a recently identified lncRNA in oncology [8] which piqued our interest. In hepatocellular carcinoma, elevated ACVR2B-AS1 expression has been reported as an independent predictor of poor prognosis, affecting both overall survival and recurrence-free survival [9]. While ACVR2B-AS1 has been identified as a tumor-associated factor in thyroid cancer, for example, a TC immune-related lncRNA prognostic model showed that ACVR2B-AS1 is upregulated in TC patients [10]. However, the specific mechanism of action of ACVR2B-AS1 in TC remains unexplored. The expression validation in tumor samples revealed significant changes in ACVR2B-AS1 expression, indicating that it may be a promising candidate for further investigation.

MicroRNAs (miRNAs) are short, non-coding RNAs, approximately 22 nucleotides in length, which regulate gene expression by binding to the 3′-UTR region of target genes, thereby inhibiting their translation or promoting their degradation [11, 12]. Research has shown that miRNAs have dual roles in tumor development and progression, functioning as both tumor suppressors and oncogenes [13]. miR-195-5p is recognized as a tumor suppressor in various cancers, including esophageal, lung, prostate, gastric, nasopharyngeal squamous cell carcinoma, and hepatocellular carcinoma, where it is downregulated and inhibits tumor cell viability, migration, and invasion by targeting multiple oncogenes [8, 14, 15]. In TC, miR-195-5p is downregulated, suggesting its potential as a biomarker for the disease [16]. lncRNAs have been shown to indirectly regulate miRNA target gene expression via the "sponge effect," where they competitively bind to miRNAs [17]. Bioinformatics analysis predicted several binding sites between lncRNA ACVR2B-AS1 and miR-195-5p, suggesting that ACVR2B-AS1 may downregulate miR-195-5p expression through this mechanism, thereby promoting TC progression. This study aimed to further explore and verify this hypothesis.

The study aimed to assess the expression profile of ACVR2B-AS1 in TC and its potential as a prognostic marker. Additionally, in vitro cell experiments were performed to investigate the molecular regulatory interactions between ACVR2B-AS1 and miR-195-5p in TC progression. This study is expected to provide new theoretical insights and practical guidance for understanding the molecular mechanism of TC.

2 Materials and methods

2.1 Inclusion of subjects

A total of 126 TC patients who underwent thyroidectomy at Central Hospital of Hengyang between June 2017 and June 2019 were enrolled in this study. Patients with prior radiotherapy or chemotherapy were excluded. The research was sanctioned by the Ethics Committee of Central Hospital of Hengyang, with participants providing informed allow.

2.2 Basic information and sample collection

Comprehensive clinical data, including age, gender, lymph node metastasis, extrathyroidal extension, TNM stage, tumor size, and differentiation, were collected from patients. Tumor and adjacent normal tissues were collected during surgery, frozen in liquid nitrogen, and stored at − 80 °C. Patients were followed for five years to gather survival information.

2.3 Cell culture

The five thyroid cancer cell lines (FTC-133, 8505C, K1, SW579, and IHH-4) [18–20] and the human thyroid cell line Nthy-ori3-1 used in this study were purchased from ECACC (Salisbury, UK). FTC-133 cells were cultured in DMEM/F-12 medium, Nthy-ori3-1, 8505C, and IHH-4 in RPMI-1640, K1 in high-glucose DMEM, and SW579 in Leibovitz's L-15, all supplemented with 10% FBS. All cultures were kept at 37 °C with 5% CO_2.

2.4 qRT-PCR

Total RNA from tissues and cells was extracted using the RNeasy Mini Kit (Qiagen, Germany) as per the instructions. RNA from lncRNA ACVR2B-AS1 and miR-195-5p was reverse-transcribed into cDNA using the PrimeScript™ RT Reagent Kit (TaKaRa, Japan) and the TaqMan MicroRNA Reverse Transcription Kit (Thermo Fisher Scientific, USA), respectively. Quantitative PCR was conducted with the SYBR premix Ex Taq II Kit (Takara Bio, Japan) on an MX3000p real-time PCR instrument. The PCR cycling conditions were as follows: initial denaturation, 95 °C for 30 s; PCR, 40 cycles of 95 °C for 5 s and 60 °C for 30 s; dissociation, 95 °C for 15 s, 60 °C for 60 s, and 95 °C for 15 s. GAPDH and U6 served as reference genes, and relative expression was determined by the $2^{-\Delta\Delta Ct}$ method.

2.5 Dual-luciferase gene reporter assay

The potential binding sites between ACVR2B-AS1 and miR-195-5p were predicted by the lncRNASNP v3 (http://gong_lab.hzau.edu.cn/lncRNASNP3/). The ACVR2B-AS1 fragment including the miR-195-5p binding site was cloned into pGL3 to generate wild-type (WT) and mutant (MT) ACVR2B-AS1 vectors. These vectors were then co-transfected with miR-195-5p mimics or inhibitors into SW579 and IHH-4 cells employing Lipofectamine 3000 (Thermo Fisher Scientific, USA). 48 h later, luciferase activity was obtained by the dual-luciferase reporter assay, with Renilla luciferase as control. The potential binding sites between FGF2 and miR-195-5p were predicted by the ENCORI (https://rnasysu.com/encori/). Dual luciferase assay for FGF2 and miR-195-5p was performed as above.

2.6 Cell transfection

Around 2×10^5 SW579 and IHH-4 cells were seeded per well in 6-well plates and cultured until reaching 70–80% confluence. siRNA targeting ACVR2B-AS1 transcripts (si-ACVR2B-AS1) and its negative control (si-NC), si-ACVR2B-AS1 with miRNA inhibitor negative control (si-ACVR2B-AS1 + inhibitor-NC), and si-ACVR2B-AS1 with miR-195-5p inhibitor (si-ACVR2B-AS1 + miR inhibitor) were mixed with Lipofectamine 3000 at room temperature for 10 min to form complexes. The complexes were introduced into the cells and incubated for 48 h. After incubation, qRT-PCR was used to quantify ACVR2B-AS1 and miR-195-5p expression. The transfection of si-FGF2 (Invitrogen, Carlsbad, USA) and miRNA relative material was performed as above. The sequence was shown in the supplementary Table S1.

2.7 Cell viability

SW579 and IHH-4 cells, after transfection, were seeded at 1×10^4 cells per well in 96-well plates. Following treatment, 10 µL of CCK-8 reagent (Solarbio, China) was added to each well and incubated for 2 h at different intervals (0, 24, 48, and 72 h). Absorbance was read at 450 nm employing a Bio-Rad microplate reader (USA).

2.8 Cell migration and cell invasion

To evaluate cell migration and invasion, Transwell assays were used. Cells were digested, counted, and suspended at 5×10^4 cells per well in 200 µL of serum-free medium, then added to the upper chamber, while the lower chamber contained 500 µL of medium with 10% FBS as a chemoattractant. For migration, cells were incubated at 37 °C with 5% CO_2 for 24 h. For invasion, Matrigel-coated chambers were used, following the same procedure. After incubation,

non-migrated or non-invaded cells were removed, and those on the lower side were fixed with 4% paraformaldehyde, stained with 0.1% crystal violet, and counted under a microscope.

2.9 Data analysis

Data analysis and visualization were performed using SPSS 23 or GraphPad Prism 9. Group differences were tested with Student's t-test or one-way ANOVA, followed by Tukey's post-hoc test, with statistical significance defined as $P < 0.05$.

3 Results

3.1 Expression of lncRNA ACVR2B-AS1 in TC tissues and cells

lncRNA ACVR2B-AS1 expression was significantly elevated in TC tissues relative to normal tissues in TC patients ($P < 0.001$, Fig. 1A), as measured by qRT-PCR. The five TG cell lines were selected to verify the ACVR2B-AS1 expression. Compared to normal human thyroid cells (Nthy-ori3-1), lncRNA ACVR2B-AS1 expression was markedly upregulated in TC cell lines (FTC-133, 8505C, K1, SW579, and IHH-4), with the most significant upregulation observed in SW579 and IHH-4 cells ($P < 0.001$, Fig. 1B).

3.2 Correlation between lncRNA ACVR2B-AS1 Expression and clinicopathological features

The relationship between ACVR2B-AS1 and clinical features was assessed to explore the clinical value. Based on the mean expression level of lncRNA ACVR2B-AS1 in the TC tissues of 126 patients (1.63), the patients were categorized into high and low expression groups. The χ^2 test revealed a significant correlation between ACVR2B-AS1 expression levels and TNM stage ($P = 0.018$) and lymph node metastasis ($P = 0.011$) (Table 1).

3.3 Correlation between lncRNA ACVR2B-AS1 expression and overall survival

Patients exhibiting high lncRNA ACVR2B-AS1 expression showed markedly reduced survival rates compared to those with low expression levels ($P = 0.016$, Fig. 1C) in Kaplan–Meier survival curves. Additionally, high lncRNA ACVR2B-AS1 expression (HR = 4.692, $P = 0.029$), positive lymph node metastasis (HR = 3.762, $P = 0.041$), and TNM stage III/IV (HR = 4.877, $P = 0.037$) were identified as independent prognostic factors for TC patients (Table 2) by cox regression analysis.

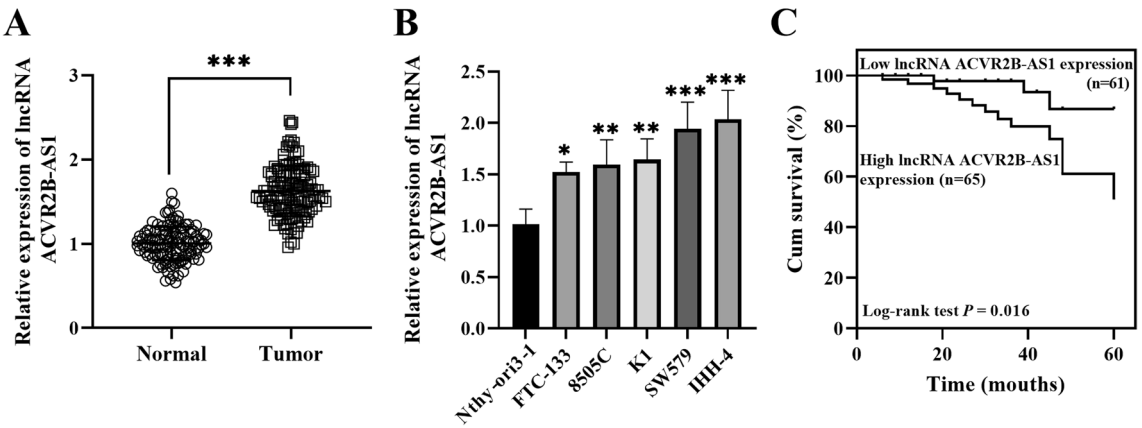

Fig. 1 The expression of lncRNA ACVR2B-AS1 in thyroid cancer tissues and cells, and the Kaplan–Meier survival curves of thyroid cancer patients. **A** The expression of ACVR2B-AS1 increased in thyroid cancer tissues compared with adjacent normal tissues by qRT-PCR (n = 125). **B** ACVR2B-AS1 expression was higher in thyroid cancer cell lines (FTC-133, 8505C, K1, SW579, and IHH-4) compared to normal thyroid cell line (Nthy-ori 3–1) by qRT-PCR (n = 5). **C** High expression of ACVR2B-AS1 was associated with poor prognosis in patients. Data are presented as the mean ± SD. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

Table 1 Association between expression of lncRNA ACVR2B-AS1 and the clinicopathological characteristics of patients

Clinicopathological characteristics	Low expression of lncRNA ACVR2B-AS1 (n = 61)	High expression of lncRNA ACVR2B-AS1 (n = 65)	*P*
Age			0.402
< 50	27	24	
≥ 50	34	41	
Gender			0.600
Male	31	30	
Female	30	35	
Extra thyroidal extension			0.228
Negative	32	41	
Positive	29	24	
Lymph node metastasis			0.011
Negative	50	40	
Positive	11	25	
Tumor size (cm)			0.106
< 4	36	29	
≥ 4	25	36	
TNM stage			0.018
I/II	46	36	
III/IV	15	29	
Differentiation			0.058
Well + moderate	42	34	
Poor	19	31	

Table 2 Multivariate Cox analysis of clinical parameters in relation to overall survival

	HR Value	95% CI	*P*
LncRNA ACVR2B-AS1	4.692	1.168–18.844	0.029
Age	1.468	0.46–4.682	0.517
Gender	1.110	0.339–3.634	0.863
Extra thyroidal extension	1.253	0.413–3.801	0.690
Lymph node metastasis	3.762	1.056–13.395	0.041
Tumor size (cm)	1.846	0.606–5.63	0.281
TNM stage	4.877	1.098–21.657	0.037
Differentiation	1.983	0.552–7.128	0.294

3.4 Evaluation of the relationship between lncRNA ACVR2B-AS1 and miR-195-5p

miR-195-5p expression was significantly lower in TC tumor tissues than in normal tissues, as measured by qRT-PCR($P < 0.001$, Fig. 2A). A significant negative correlation was observed between the relative expression levels of lncRNA ACVR2B-AS1 and miR-195-5p in TC tumor tissues according to the Pearson correlation analysis ($r = -0.773$, $P < 0.001$, Fig. 2B). Additionally, in TC cell lines (FTC-133, 8505C, K1, SW579, and IHH-4), miR-195-5p levels were also notably reduced compared to Nthy-ori3-1 cells ($P < 0.001$, Fig. 2C). SW579 and IHH-4 cells, with relatively low miR-195-5p expression and significantly elevated ACVR2B-AS1 expression, were chosen for further experiments.

The association between miR-195-5p and ACVR2B-AS1 was further verified. Binding sites between miR-195-5p and ACVR2B-AS1 were identified employing the lncRNASNP v3 database (Fig. 2D). As shown in the dual-luciferase gene reporter assay, in SW579 (Fig. 2E) and IHH-4 (Fig. 2F) cells, miR-195-5p overexpression notably suppressed the luciferase activity of WT ACVR2B-AS1, while its knockdown enhanced the activity ($P < 0.001$). However, the luciferase activity of MT ACVR2B-AS1 remained unaffected ($P > 0.05$), confirming a targeted relationship between lncRNA ACVR2B-AS1 and miR-195-5p.

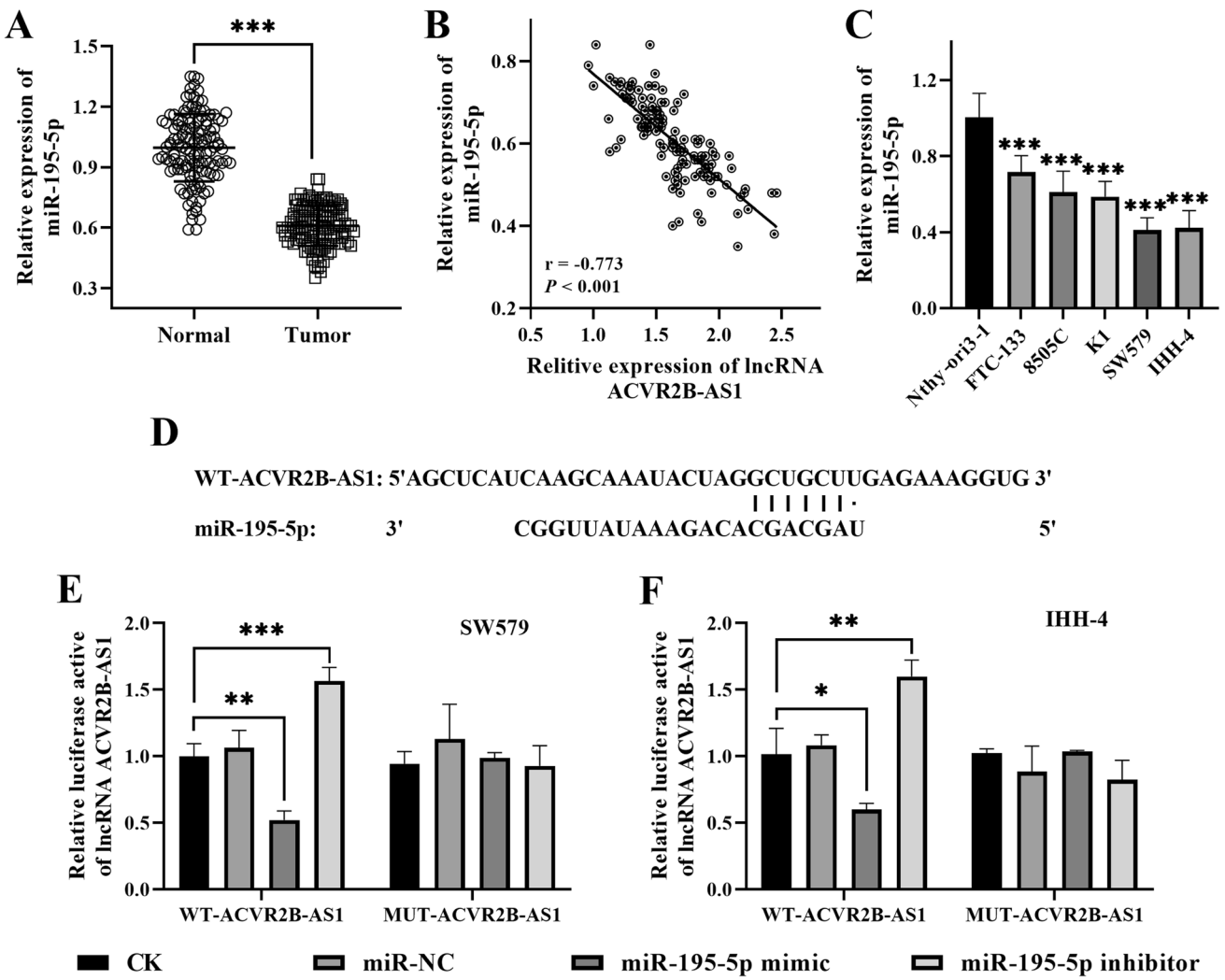

Fig. 2 Evaluation of the relationship between lncRNA ACVR2B-AS1 and miR-195-5p. **A** miR-195-5p expression was decreased in thyroid cancer tissues compared with adjacent normal tissues by qRT-PCR (n = 125). **B** Pearson analysis revealed that the significant negative correlation between the relative expression levels of ACVR2B-AS1 and miR-195-5p in TC tissues (n = 125). **C** The expression of miR-195-5p decreased in thyroid cancer cell lines (FTC-133, 8505C, K1, SW579, and IHH-4) compared with normal thyroid cell line (Nthy-ori 3–1) by qRT-PCR (n = 5). **D** Binding sites between miR-195-5p and ACVR2B-AS1 were identified using the lncRNASNP v3 database. **E and F** Dual-luciferase reporter gene assay for wild-type and mutant ACVR2B-AS1 luciferase vectors in SW579 cells **E** and IHH-4 cells **F** transfected with miR-195-5p mimic or miR-195-5p inhibitor (n = 3). Data are presented as the mean ± SD. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

3.5 Evaluation of the regulatory interaction between lncRNA ACVR2B-AS1 and miR-195-5p

Next, the mechanism of ACVR2B-AS1 in TC was investigated. The transfection efficiency was tested in the cell experiment. In SW579 (Fig. 3A) and IHH-4 (Fig. 3B) cells, knockdown of lncRNA ACVR2B-AS1 significantly reduced lncRNA ACVR2B-AS1 expression ($P < 0.001$), while miR-195-5p knockdown did not affect lncRNA ACVR2B-AS1 expression ($P > 0.05$). Conversely, in SW579 (Fig. 3C) and IHH-4 (Fig. 3D) cells, silencing lncRNA ACVR2B-AS1 led to a marked increase in miR-195-5p expression ($P < 0.001$), which was notably reversed by co-knockdown of ACVR2B-AS1 and miR-195-5p ($P < 0.05$). These results confirm that the transfection was successful.

Knockdown of ACVR2B-AS1 greatly inhibited the viability of SW579 (Fig. 3E) and IHH-4 (Fig. 3F) cells ($P < 0.001$), and significantly reduced the migration (Fig. 3G and H) and invasion (Fig. 3I and J) of SW579 and IHH-4 cells ($P < 0.001$). However, simultaneous knockdown of lncRNA ACVR2B-AS1 and miR-195-5p reversed the effects of ACVR2B-AS1 silencing on TC cell viability, migration, and invasion.

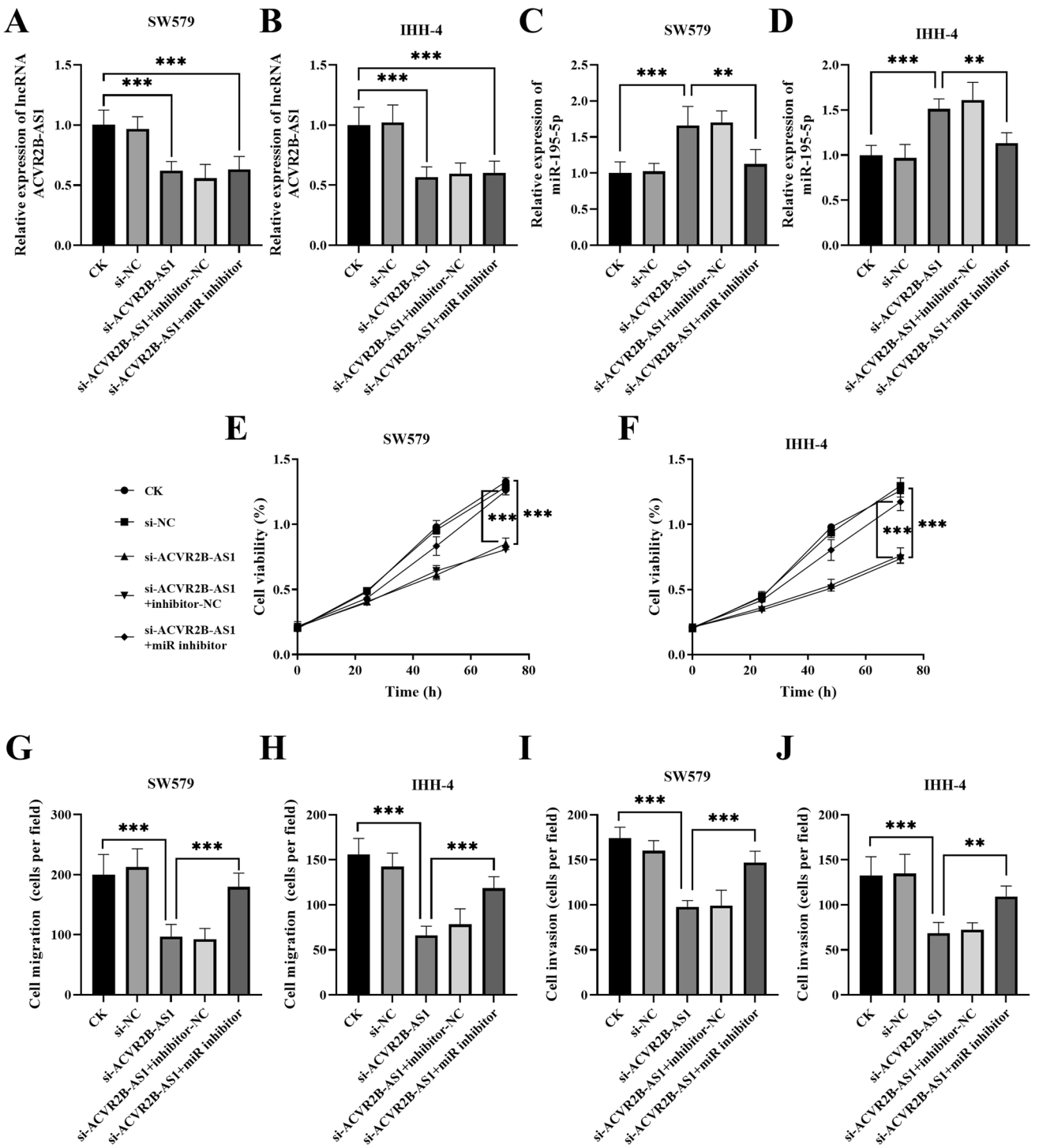

Fig. 3 lncRNA ACVR2B-AS1 regulates miR-195-5p, affecting the viability, migration, and invasion of SW579 and IHH-4 cells. **A and B** In SW579 (**A**) and IHH-4 (**B**) cells, knockdown of ACVR2B-AS1 significantly reduced ACVR2B-AS1 expression, whereas knockdown of miR-195-5p had no effect on ACVR2B-AS1 expression, as determined by qRT-PCR (n = 5). **C and D** In SW579 (**C**) and IHH-4 (**D**) cells, knockdown of ACVR2B-AS1 significantly increased miR-195-5p expression, and this effect was significantly reversed by simultaneous knockdown of both ACVR2B-AS1 and miR-195-5p, as measured by qRT-PCR (n = 3). **E–J** Knockdown of ACVR2B-AS1 significantly inhibited the viability of SW579 (**E**) and IHH-4 (**F**) cells, and reduced the migration of SW579 (**G**) and IHH-4 (**H**) cells, as well as invasion of SW579 (**I**) and IHH-4 (**J**) cells, and this effect was significantly reversed by simultaneous knockdown of both ACVR2B-AS1 and miR-195-5p, as measured by CCK-8 and Transwell (n = 5). Data are presented as the mean ± SD. **, $P < 0.01$; ***, $P < 0.001$

3.6 Evaluation of the relationship between miR-195-5p and FGF2

FGF2 expression was higher in TC tissues than in normal tissues in the TCGA dataset (Fig. 4A). In TC cell lines (K1, SW579, and IHH-4), FGF2 levels were also notably increased compared to Nthy-ori3-1 cells ($P < 0.01$, Fig. 4B). In SW579 (Fig. 4C) and IHH-4 (Fig. 4D) cells, knockdown of miR-195-5p significantly increased FGF2 expression, while FGF2 expression was notably reversed by co-knockdown of ACVR2B-AS1 and miR-195-5p ($P < 0.05$). Binding sites between miR-195-5p and FGF2 were predicted by the ENCORI (Fig. 4E). In SW579 (Fig. 4F) and IHH-4 (Fig. 4G) cells, miR-195-5p overexpression notably suppressed the luciferase activity of WT FGF2, while its knockdown enhanced the activity ($P < 0.05$). However, the luciferase activity of MT FGF2 remained unaffected ($P > 0.05$), confirming a targeted relationship between miR-195-5p and FGF2.

4 Discussion

The role of lncRNAs in regulating disease progression through various molecular mechanisms has been extensively studied [21]. Several lncRNAs have been identified as dysregulated in tumor tissues from TC patients, indicating their crucial regulatory roles in TC progression [22, 23]. lncRNA ARAP1-AS1 is upregulated in TC cell lines and tissues, promoting TC progression by interacting with the miR-516b-5p/PDE5A axis [24]. Similarly, lncRNA CDKN2B-AS1 is upregulated in TC samples, with its overexpression potentially reflecting an active immune microenvironment [25]. A newly identified lncRNA, ACVR2B-AS1, was found to be upregulated in TC patient tissues in a study that constructed a TC-related lncRNA

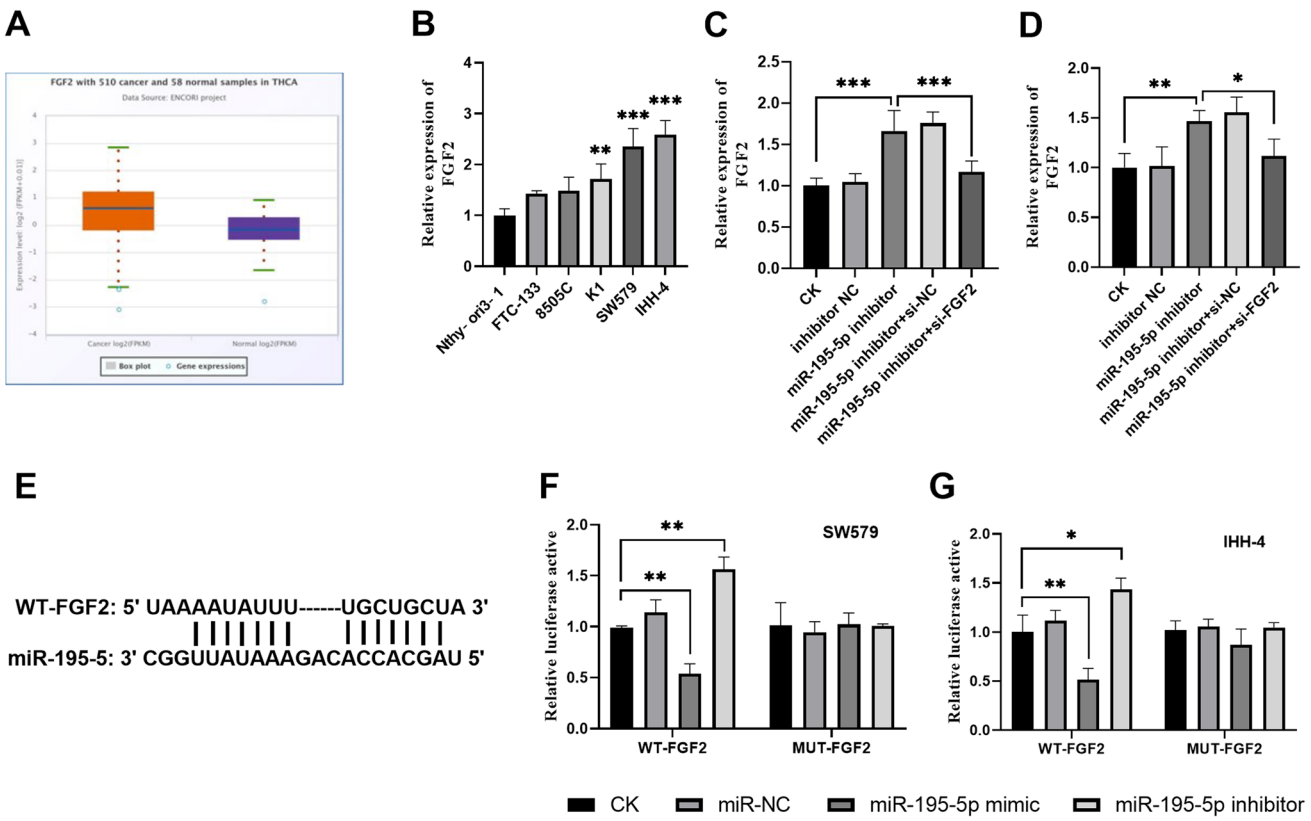

Fig. 4 Evaluation of the relationship between miR-195-5p and FGF2. **A** FGF2 expression was increased in thyroid cancer (n = 510) compared with normal samples (n = 58) in TCGA datasets. **B** The expression of FGF2 increased in thyroid cancer cell lines (K1, SW579, and IHH-4) compared with normal thyroid cell line (Nthy-ori 3–1) by qRT-PCR (n = 5). **C and D** In SW579 (**C**) and IHH-4 (**D**) cells, knockdown of miR-195-5p significantly increased FGF2 expression, and this effect was significantly reversed by simultaneous knockdown of both miR-195-5p and FGF2, as measured by qRT-PCR (n = 5). **D** Binding sites between miR-195-5p and FGF2 were identified using the ENCORI database. **E and F** Dual-luciferase reporter gene assay for wild-type and mutant FGF2 luciferase vectors in SW579 cells (**E**) and IHH-4 cells (**F**) transfected with miR-195-5p mimic or miR-195-5p inhibitor (n = 3). Data are presented as the mean ± SD. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

prognostic model [10], aligning with the findings of the present study. Lymph node metastasis is a critical indicator of tumor progression, as it reflects the spread of tumor cells through the lymphatic system, signifying a failure of local control and potential for distant metastasis [26]. TNM stage serves as the primary metric for assessing tumor severity. Advanced-stage tumors (TNM stage III or IV) are often linked to larger tumor size, extensive local invasion, lymph node involvement, and distant metastasis [27]. Both lymph node metastasis and high TNM stage are indicative of late-stage disease, poor prognosis, and significantly reduced survival rates. The results suggest that ACVR2B-AS1 is highly upregulated in TC tissues and cell lines, and this upregulation is strongly associated with adverse clinicopathological characteristics, including lymph node metastasis and high TNM stage. Previous studies have demonstrated that abnormal lncRNAs expression in various cancers is closely associated with their oncogenic or tumor-suppressive functions [28]. For instance, lncRNA RMRP has been identified as an oncogene in bladder cancer, with its high expression significantly correlated with poor prognosis [29]. This aligns with the findings in TC patients in the current study, suggesting that ACVR2B-AS1 may act as an oncogenic factor in TC progression, promoting malignant behaviors through multiple mechanisms. These findings further confirmed that elevated ACVR2B-AS1 expression serves as an independent prognostic factor for decreased survival in TC patients, indicating its potential as a biomarker for TC prognosis.

miRNAs have been widely studied as key regulators in tumorigenesis [13]. miR-195-5p, a well-established tumor suppressor, has been demonstrated to play a critical role in several types of cancer [14], including TC [16], where its expression is downregulated. The results of this study verify that miR-195-5p is reduced in both TC tumor tissues and cell lines. Current research suggests that lncRNAs influence the progression of various diseases by "sponging" miRNAs [30, 31]. This study reveals a strong inverse correlation between ACVR2B-AS1 and miR-195-5p expression in TC tumor tissues, supported by evidence of a targeted interaction between them. These findings suggest that ACVR2B-AS1 may facilitate TC progression by regulating miR-195-5p regulation. Studies have shown that the interaction between lncRNAs and miRNAs not only affects gene transcription and translation but also influences cell proliferation, migration, and invasion through complex regulatory networks, ultimately driving disease progression [32–34]. The study results indicate that ACVR2B-AS1 negatively regulates miR-195-5p. Furthermore, co-knockdown of ACVR2B-AS1 and miR-195-5p reverses the suppressive effects of ACVR2B-AS1 silencing on TC cell viability, migration, and invasion, providing further evidence of their functional interplay. Previous research has shown that miR-195-5p decreases TC cell proliferation by modulating the p21/cyclin D1 axis [35]. Linc00210 has been identified as a miR-195-5p sponge, negating its capacity to reduce the expression of IGF1R and block PI3K/Akt pathway activation, thus impacting TC progression [16]. These insights suggest that ACVR2B-AS1 may downregulate miR-195-5p expression via a "sponge effect". This regulation likely influences key proteins and signaling pathways, driving the aggressive behavior of TC cells.

The target genes of miR-195-5p were predicted to explore the potential molecular mechanisms in TC. Potential binding sites between miR-195-5p and FGF2 were identified. FGF2 plays a critical regulatory role in tumors, for example, increased FGF2 expression promotes immune cell infiltration and is associated with poor prognosis in thyroid cancer [36]. MiR-195 has been shown to influence tumor growth and metastasis in papillary thyroid carcinoma cell lines by targeting FGF2 [37]. The expression level of FGF2 was significantly upregulated in TC tissues and cell lines. Therefore, lncRNA ACVR2B-AS1 inhibits miR-195-5p and regulates the proliferation and metastasis of TC cells through FGF2, contributing to the progression of TC.

The cell lines FTC-133, 8505C, K1, SW579, and IHH-4 are derived from thyroid gland follicular carcinoma, thyroid gland anaplastic carcinoma, thyroid gland papillary carcinoma, thyroid gland squamous cell carcinoma, and thyroid gland papillary carcinoma respectively. The two TC cell lines, SW579 and IHH-4, were selected based on the findings of the present study. These two cell lines represent different cell line subtypes, which helps to highlight the broader relevance of our findings across diverse tumor types. The use of these cell lines aligns with the histological diversity observed in our patient population, ensuring that our experimental models are associated with clinical reality.

This study represents the first systematic exploration of the clinical significance and molecular mechanisms of ACVR2B-AS1 in TC; however, certain limitations must be acknowledged. Although we used a cell model to investigate the mechanisms and demonstrate that ACVR2B-AS1 regulates tumor progression via the miR-195-5p/FGF2 axis, the in vitro environment remains limited and static, unable to replicate the complex and dynamic physiological conditions of in vivo systems. In vivo experiments are crucial for bridging the gap between basic research and clinical applications. Due to limitations in experimental conditions and funding, this study did not include animal experiments. In future studies, animal models will be incorporated to further assess and validate the role of ACVR2B-AS1 in TC. Additionally, while this study mainly focused on the relationship between ACVR2B-AS1 and miR-195-5p, lncRNAs typically exert their biological functions by regulating multiple miRNAs or proteins. Therefore, further exploration of other regulatory pathways involving ACVR2B-AS1 will be essential for a comprehensive understanding of its role in TC.

5 Conclusion

lncRNA ACVR2B-AS1 was observed to be significantly upregulated in TC and was closely associated with adverse clinical features and prognosis. It was demonstrated to enhance TC cell viability, migration, and invasion through the regulation of miR-195-5p expression. These findings provide new theoretical foundations and potential targets for molecular mechanism research and personalized treatment of TC.

Author contributions Tianshi Qin, Chengqiang Lei, Huayi Zhang, Henghua Xiao and Manlong Long carried out the concepts, design and definition of intellectual content, Henghua Xiao, Jun Yang, Qiong Luo, Lingli Hu and Fang Chen provided assistance for data acquisition, data analysis and statistical analysis. All authors performed the experiment, and draft of the manuscript. Manlong Long, Tianshi Qin, Chengqiang Lei and Huayi Zhang revised the manuscript critically for important intellectual content. All authors have read and approved the content of the manuscript.

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Code availability Not applicable.

Declarations

Competing interests The authors declare no competing interests.

References

1. Guo K, Qian K, Shi Y, Sun T, Wang Z. LncRNA-MIAT promotes thyroid cancer progression and function as ceRNA to target EZH2 by sponging miR-150-5p. Cell Death Dis. 2021;12(12):1097.
2. Zhang X, Zhang X, Jia Q, Li H, Ma R, Yang G, et al. LncRNA CALML3-AS1 suppresses papillary thyroid cancer progression via sponging miR-20a-5p/RBM38 axis. BMC Cancer. 2022;22(1):344.
3. Nabhan F, Dedhia PH, Ringel MD. Thyroid cancer, recent advances in diagnosis and therapy. Int J Cancer. 2021;149(5):984–92.
4. Seib CD, Sosa JA. Evolving understanding of the epidemiology of thyroid cancer. Endocrinol Metab Clin North Am. 2019;48(1):23–35.
5. Zeng Z, Tang S, Chen L, Hou H, Liu Y, Li J. LncRNA HAGLROS contribute to papillary thyroid cancer progression by modulating miR-206/HMGA2 expression. Aging. 2023;15(24):14930–44.
6. Luzón-Toro B, Fernández RM, Martos-Martínez JM, Rubio-Manzanares-Dorado M, Antiñolo G, Borrego S. LncRNA LUCAT1 as a novel prognostic biomarker for patients with papillary thyroid cancer. Sci Rep. 2019;9(1):14374.
7. Tan YT, Lin JF, Li T, Li JJ, Xu RH, Ju HQ. LncRNA-mediated posttranslational modifications and reprogramming of energy metabolism in cancer. Cancer Commun. 2021;41(2):109–20.
8. Hu S, Zhou H, Zhao X, Qian F, Jin C. MiR-195-5p suppresses gastric adenocarcinoma cell progression via targeting OTX1. Histol Histopathol. 2023;38(6):659–68.
9. Nie Y, Jiao Y, Li Y, Li W. Investigation of the clinical significance and prognostic value of the lncRNA ACVR2B-As1 in liver cancer. Biomed Res Int. 2019;2019:4602371.
10. Li Z, Wang H, Deng X, Zhang J, Wang L, Tang W, et al. Construction and validation of an immune-related lncRNA prognosis model for thyroid cancer. Comb Chem High Throughput Screen. 2022;25(13):2217–27.
11. Chen L, Heikkinen L, Wang C, Yang Y, Sun H, Wong G. Trends in the development of miRNA bioinformatics tools. Brief Bioinform. 2019;20(5):1836–52.
12. Lu TX, Rothenberg ME. MicroRNA. J Allergy Clin Immunol. 2018;141(4):1202–7.
13. Hill M, Tran N. miRNA interplay: mechanisms and consequences in cancer. Dis Models Mech. 2021. https://doi.org/10.1242/dmm.047662.

14. Xu Q, Xu JL, Chen WQ, Xu WX, Song YX, Tang WJ, et al. Roles and mechanisms of miR-195–5p in human solid cancers. Biomed Pharmacother Biomed Pharmacother. 2022;150:112885.
15. Liu X, Liu Z, Liu Y, Wang N. ATG9A modulated by miR-195-5p can boost the malignant progression of cervical cancer cells. Epigenetics. 2023;18(1):2257538.
16. Du P, Liu F, Liu Y, Shao M, Li X, Qin G. Linc00210 enhances the malignancy of thyroid cancer cells by modulating miR-195-5p/IGF1R/Akt axis. J Cell Physiol. 2020;235(2):1001–12.
17. Jiang L, Wu Z, Meng X, Chu X, Huang H, Xu C. LncRNA HOXA-AS2 facilitates tumorigenesis and progression of papillary thyroid cancer by modulating the miR-15a-5p/HOXA3 Axis. Hum Gene Ther. 2019;30(5):618–31.
18. Landa I, Pozdeyev N, Korch C, Marlow LA, Smallridge RC, Copland JA, et al. Comprehensive genetic characterization of human thyroid cancer cell lines: a validated panel for preclinical studies. Clin Cancer Res. 2019;25(10):3141–51.
19. Yang X, Li W, Han X, Wang J, Dai J, Ye X, et al. Apatinib weakens proliferation, migration, invasion, and angiogenesis of thyroid cancer cells through downregulating pyruvate kinase M2. Sci Rep. 2024;14(1):879.
20. Zhou Z, Li Y, Yan X, Wang X, Chen S, Xiao J. Characteristics of a thyroid carcinoma cell line derived from spinal metastasis. 2016. Biosci Rep. https://doi.org/10.1042/BSR20160403.
21. Bridges MC, Daulagala AC, Kourtidis A. LNCcation: lncRNA localization and function. J Cell Biol. 2021. https://doi.org/10.1083/jcb.202009045.
22. Song B, Tian L, Zhang F, Lin Z, Gong B, Liu T, et al. A novel signature to predict thyroid cancer prognosis and immune landscape using immune-related LncRNA pairs. BMC Med Genomics. 2022;15(1):183.
23. Javed Z, Ahmed Shah F, Rajabi S, Raza Q, Iqbal Z, Ullah M, et al. LncRNAs as potential therapeutic targets in thyroid cancer. Asian Pacific J Cancer Prevent APJCP. 2020;21(2):281–7.
24. Du Q, Huang L, Guo W. LncRNA ARAP1-AS1 targets miR-516b-5p/PDE5A axis to facilitate the progression of thyroid cancer. Anticancer Drugs. 2023;34(6):735–46.
25. Xue C, Yi C, Lin H, Zhao J, Yuan J, Chen Y, et al. lncRNA CDKN2B-AS1 could be an indicator to identify prognosis and status of immune microenvironment in thyroid cancer. Dis Markers. 2022;2022:4317480.
26. Peng JM, Su YL. Lymph node metastasis and tumor-educated immune tolerance: potential therapeutic targets against distant metastasis. Biochem Pharmacol. 2023;215: 115731.
27. Pang L, Wang J, Fan Y, Xu R, Bai Y, Bai L. Correlations of TNM staging and lymph node metastasis of gastric cancer with MRI features and VEGF expression. Cancer Biomarkers Sect A Dis Markers. 2018;23(1):53–9.
28. Grammatikakis I, Lal A. Significance of lncRNA abundance to function. Mamm Genome Off J Int Mamm Genome Soc. 2022;33(2):271–80.
29. Cao HL, Liu ZJ, Huang PL, Yue YL, Xi JN. lncRNA-RMRP promotes proliferation, migration and invasion of bladder cancer via miR-206. Eur Rev Med Pharmacol Sci. 2019;23(3):1012–21.
30. Yang S, Wang Y, Lin Y, Shao D, He K, Huang L. LncMirNet: predicting LncRNA-miRNA interaction based on deep learning of ribonucleic acid sequences. Molecules. 2020. https://doi.org/10.3390/molecules25194372.
31. Liao J, Wang J, Liu Y, Li J, Duan L. Transcriptome sequencing of lncRNA, miRNA, mRNA and interaction network constructing in coronary heart disease. BMC Med Genomics. 2019;12(1):124.
32. Bhattacharjee R, Prabhakar N, Kumar L, Bhattacharjee A, Kar S, Malik S, et al. Crosstalk between long noncoding RNA and microRNA in cancer. Cell Oncol. 2023;46(4):885–908.
33. López-Urrutia E, Montes LPB, Cervantes DLDG, Perez-Plasencia C. Parra ADCJFiO crosstalk between long non-coding RNAs, Micro-RNAs and mRNAs: deciphering molecular mechanisms of master regulators in cancer. Front Oncol. 2019;9:669.
34. Wheeler BD, Gagnon JD, Zhu WS, Muñoz-Sandoval P, Wong SK, Simeonov DS, et al. The lncRNA Malat1 inhibits miR-15/16 to enhance cytotoxic T cell activation and memory cell formation. Elife. 2023;12:RP87900.
35. Liu D, Li P, Wang X, Wang W. hsa-miR-195-5p inhibits cell proliferation of human thyroid carcinoma cells via modulation of p21/cyclin D1 axis. Trans Cancer Res. 2020;9(9):5190–9.
36. Chen H, Du X. Increased FGF2 expression promotes immune cell infiltration and correlates with an unfavorable prognosis in thyroid cancer. Heliyon. 2024;10(11): e32272.
37. Yin Y, Hong S, Yu S, Huang Y, Chen S, Liu Y, et al. MiR-195 inhibits tumor growth and metastasis in papillary thyroid carcinoma cell lines by targeting CCND1 and FGF2. Int J Endocrinol. 2017;2017:6180425.