

Circ0005654 as a new biomarker of thyroid cancer interacting with SP1 to influence the prognosis

A case-control study

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Abstract

Circular RNAs (CircRNAs) have been reported to play key roles in the progression of various cancers, including thyroid cancer (TC). Transcription factor 1 (SP1) promotes the development of thyroid cancer. This study aims at investigating the expression level of Circ0005654 in combination with Transcription factor1 (SP1) in patients with TC for diagnostic and therapeutic purposes. A total of 76 patients with thyroid cancer underwent radical surgery. Intraoperatively, thyroid cancer tissues and paired adjacent tissues and the corresponding clinicopathological data were collected. The expression of SP1 and β -catenin in thyroid cancer and adjacent tissues was determined by immunohistochemistry (IHC) while the Circ0005654 expression level was measured by semiquantitative real-time polymerase chain reaction (sqRT-PCR). Then, we compared the variability of Circ0005654, SP1, and Wnt/ β -catenin expression in cancerous and adjacent tissues and determined the relationship between the correlation analysis and the clinicopathological features of the thyroid cancer patients. The diagnostic value of Circ0005654 in thyroid cancer tissues was analyzed with the help of the receiver operating characteristic (ROC) curve, counting the 3-year postoperative survival rate, and analyzing the effect of Circ0005654 and SP1 protein levels on the 3-year survival rate of the patients. sqRT-PCR showed that the expression level of Circ0005654 in thyroid cancer tissue was significantly higher than that of adjacent tissues. The area under the ROC of Circ0005654 was 0.9553, 95% confidence interval: (0.9211–0.9895) with a cutoff value of 0.7895, a sensitivity of 92.11%, and a specificity of 86.84%. The IHC results showed that the expression level of SP1, β -catenin, and Wnt was higher in cancer tissues than in adjacent tissues; Circ0005654, SP1, Wnt/ β -catenin expression levels were associated with tumor diameter, lymph node metastasis, TNM stage, and envelope invasion (all $P < .05$). According to the Circ0005654 expression level in thyroid cancer tissue, the 3-year survival rate of the high expression group was 77.5% and 94.4% in the low expression group with a statistically significant difference; the 3-year survival rate of SP1 positive and negative patients was 78.6% and 100%, respectively, with the data being significantly different. Circ0005654 may serve as a potential biomarker for thyroid cancer diagnosis and may be involved in the development of thyroid cancer.

Abbreviations: IHC = immunohistochemistry, ROC = receiver operating characteristic, SP1 = transcription factor1, sqRT-PCR = semiquantitative real-time polymerase chain reaction, TC = thyroid cancer.

Keywords: circular RNA, thyroid cancer, transcription factor

1. Introduction

Circular RNA is involved in the occurrence and development of various malignancies^[1–5] one of which is thyroid cancer (TC).^[6–12] A study^[13] shows that Circ0005654 can serve as a new early biomarker for gastric cancer and that it promotes gastric cancer

cell invasion and metastasis mainly by regulating Transcription factor1 (SP1) in gastric cancer tissues.^[14] A further study^[15] shows that SP1 is abnormally expressed in TC and participates in its development. Based on the above studies, we speculate that Circ0005654 and SP1 are abnormally expressed in TC and are closely related to the occurrence and development of TC.

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The authors have no conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics statement: All procedures performed in the studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Ethics Committee of Lu'an Hospital of Anhui Medical University and the patient's family members were informed of the relevant information. Informed consent was obtained from all participants.

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Therefore, the present study aimed at investigating the expression and clinical significance of Circ0005654 and SP1 in cancer tissues of TC patients.

2. Data and methods

2.1. General information

We selected 76 TC patients, aged 18 to 82 years, from the Affiliated Hospital of West Anhui Health Vocational College and Lu'an Hospital in the time frame between January 2017 to July 2019; TNM stage was: 22,32,12, I and 8; 19 and 57 according to the 2017 Cancer Federation (AJCC) criteria. Inclusion criteria were as follows: patients did not receive any other anti-cancer therapy before surgery; all tissue specimens were confirmed by intraoperative freezing or postoperative pathology. Exclusion criteria were as follows: patients suffered from dysfunction of the heart, liver, kidney, and other important organs; patients suffered from additional tumors or malignant diseases; Postoperative pathology was identified as a patient with benign nodules. This study was approved by the Ethics Committee of the Affiliated Hospital of West Anhui Health Vocational College and Lu'an Hospital of Anhui Medical University.

2.2. Methods

2.2.1. Immunohistochemistry (IHC) analysis. During the operation, thyroid cancer tissues and corresponding adjacent tissues were collected and quickly placed in liquid nitrogen. Then, the tissues were embedded in paraffin for sectioning (4 μm continuous sections), fully baked, and placed in citrate buffer for 10 min. The sections were incubated with monoclonal antibodies against SP1 and Wnt/β-catenin for 24 hours with a concomitant incubation with secondary antibodies for 1 hour. After diaminobenzidine staining, the sections were counterstained with hematoxylin. SP1 and Wnt/β-catenin stainings were evaluated under a microscope. Staining result and evaluation: we randomly selected 5 fields of view and scored the colored parts of the cells: unstained cells were scored 0; light yellow cells were scored 1, dark yellow cells were scored as 2, and brown cells were scored as 3. According to the score results, the samples were divided into 2 groups: a score of <2 was evaluated as being negative and was set as a low expression level sample; a score of 2 was evaluated as positive, with a high expression level.

2.2.2. Semiquantitative real-time quantitative polymerase chain reaction (sqRT-PCR) detection. Using RNA extraction methods (produced by Servicebio), total RNA from thyroid cancer tissues was isolated and quality tested. According to the Servicebio® protocol, the total RNA was reverse transcribed with the help of an RT First Strand cDNA Synthesis kit into template cDNA. The Gentier 96E/96R fully automatic medical PCR analysis system (produced by Xi'an Tianlong Technology Co., Ltd.) was used for the PCR. The total reaction volume was 10 μL and the experiment was performed according to the qRT-PCR kit instructions. The reaction conditions were: 95°C pre-denaturation for 30 seconds; then 95°C denaturation for 15 seconds, 60°C annealing for 30 seconds, and 60°C extension for 30 seconds, for a total of 40 cycles. For relative quantification of Circ0005654 expression, we used Glyceraldehyde Triphosphate Dehydrogenase as the internal reference and the 2^{-ΔΔC_t} method to calculate the relative expression of the genes. The primer sequences are shown in Table 1.

2.3. Statistical methods

Data were analyzed using the SPSS19.0 statistical software. Count data were expressed as number of cases (n, %), using the 4-grid table χ² test; the quantitative data are expressed as

Table 1

Primer sequences of circ0005654 and GAPDH.

Gene name	Upstream primer sequence (5'-3')	Downstream primer sequence (3'-5')
Circ0005654	ACATTCGTACTCA-CACCAGGGAG	ATCTTTCTATGTTCT-GTAGGCTTG
GAPDH	GGAAGCTTGTGCAT-CAATGGAATC	TGATGACCCCTTTTG-GCTCCC

GAPDH = Glyceraldehyde triphosphate dehydrogenase.

mean ± standard deviation (X̄±s). Comparisons between the 2 groups were made using an independent sample *t* test, the receiver operating characteristic (ROC) was employed to test the value of Circ0005654 in the diagnosis of thyroid cancer, and the Kaplan–Meier test was used to analyze the relationship between Circ0005654, SP1 expression, and the 3-year survival of TC patients. Values displaying a *P* < .05 were considered as being statistically significantly different.

3. Results

3.1. Circ0005654 expression levels in thyroid cancer and adjacent tissues

The relative expression levels of Circ0005654 in thyroid cancer tissues as measured by sqRT-PCR, showed that the expression level in cancer tissues was significantly higher than that of adjacent tissues (2.11 ± 0.59 vs 0.67 ± 0.56), *t* = 15.4, *P* < .0001, shown in Figure 1.

3.2. The diagnostic value of the Circ0005654 expression level in thyroid cancer

The area under the ROC curve for TC diagnosis by ROC analysis could be determined to be 0.9553 (95% confidence interval: 0.9211–0.9895), with a cutoff value of 0.7895, a sensitivity of 92.11%, and a specificity of 86.84%, see Figure 2.

3.3. Expression of SP1 and Wnt/β-catenin in thyroid cancer and adjacent tissues

By IHC staining, SP1 and Wnt/β-catenin expression could be shown to be strongly positive in cancer tissues with SP1 that

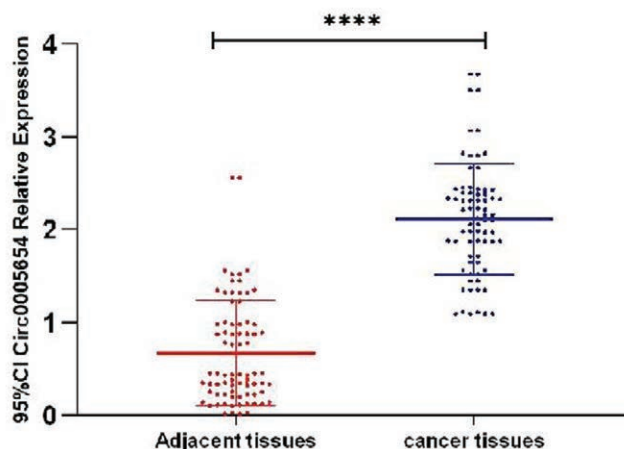


Figure 1. The relative expression levels of Circ0005654 in thyroid cancer tissues and adjacent tissues.

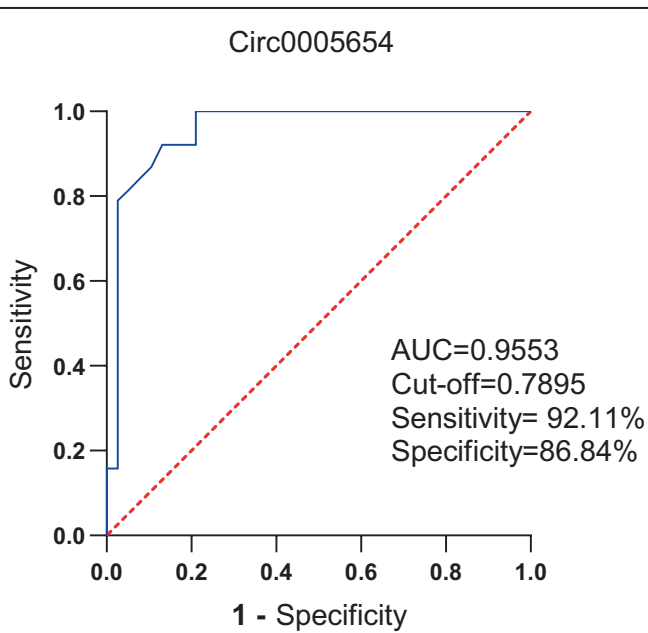


Figure 2. The diagnostic value of the Circ0005654 expression level in thyroid cancer.

mainly localized in the nucleus and cytoplasm. The positive expression rate of SP1 in adjacent thyroid carcinoma tissues was 18.0%, which was significantly lower than that of 90.7% in cancer tissues ($\chi^2 = 159.61, P < .001$). The expression of β -catenin and Wnt in cancer tissues was also higher than that of adjacent tissues with a positive rate of 57.9% (44/76) versus 13.2% (10/76) and 60.5% (46/76) versus 14.5% (11/76), respectively, which was statistically significant ($\chi^2 = 33.203$ vs $\chi^2 = 34.386, P$ in both $<.001$), see Figure 3.

3.4. The relationship between the Circ0005654, SP1, and Wnt/ β -catenin expression status and the clinicopathological characteristics of TC patients

With the median value of TC tissue Circ0005654 (2.12), thyroid cancer patients were divided into a high expression group (40 cases) and a low expression group (36 cases). Circ0005654, SP1, and Wnt/ β -catenin expression were found to be associated with tumor diameter, lymph node metastasis,

TNM stage, and capsule invasion in TC patients as judged by correlation to the clinicopathological data ($P < .05$). See Table 2.

3.5. The survival rate of thyroid cancer

The relationship between Circ0005654, SP1 expression levels in thyroid cancer tissues and survival rate was analyzed by Kaplan–Meier. The cumulative 3-year-survival rate was performed by telephone or outpatient follow-up, beginning from the date of discharge and lasting for a period of 3 years. The 3-year cumulative survival rates of Circ0005654 and SP1 patients with different expression levels in thyroid cancer tissues are shown in Figure 4 and Table 3.

According to Table 3, the 3-year cumulative survival rate of the Circ0005654 high expression group in thyroid cancer tissue was lower than the low expression group as determined by the Kaplan–Meier test ($P < .05$). As well, the 3-year cumulative survival rate of the SP1 high expression group was lower as determined by a Log-Rank test ($P < .05$).

4. Discussion

Thyroid cancer is a common endocrine malignancy with an incidence of about 1% of all systemic malignancies. The main diagnostic basis for TC comprises a detailed clinical history data collection, color ultrasound, and ultrasound-guided fine-needle aspiration cytology biopsy. Acupuncture biopsy is one of the most important means to judge the nature of thyroid nodules, but its positive predictive value and sensitivity are still affected by many factors. To improve the diagnostic level and the therapeutic outcome, it is necessary to find new diagnostic and early predictors for this disease one of which could be CircRNA. It was found that CircRNA is involved in all aspects of the tumor progression process.^[16] Earlier studies suggested that CircRNAs are fragments generated by mis-splicing of mRNA precursors.^[8] Increasing numbers of CircRNA members are being found within different organisms due to rapid advances in gene-chip technology, high-throughput sequencing, and bioinformatics. The CircRNAs exhibit a closed-loop structure.^[17] It can stably exist in various tissues and peripheral blood and, moreover, is highly disease-specific.^[8] CircRNAs have functions related to RNA binding proteins where they regulate gene expression and participate in protein translation and finally, are closely related to the clinicopathological characteristics of thyroid cancer.^[18] Therefore, these molecules might be of interest as potential targets for the intervention and treatment of thyroid cancer. This study was intended to verify the role of Circ0005654

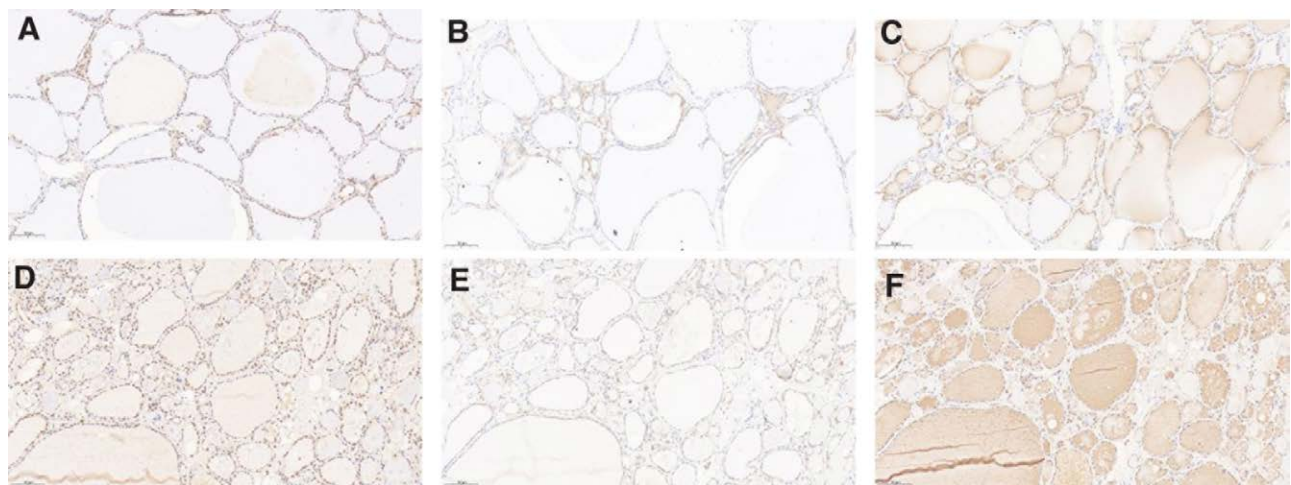


Figure 3. The expression of SP1 and Wnt/ β -catenin in thyroid cancer and adjacent tissues. SP1 = transcription factor1.

Table 2

Relationship between clinicopathological factors of TC patients and levels of Circ0005654, SP1, β -catenin, and Wnt, (n).

Characteristics	Circ0005654		SP1				β -catenin				Wnt					
	High	Low	Positive		Negative		Positive		Negative		Positive		Negative			
	(n = 40)	(n = 36)	χ^2	P	(n = 56)	(n = 20)	χ^2	P	(n = 44)	(n = 32)	χ^2	P	(n = 46)	(n = 30)	χ^2	P
Age (yr)			2.833	.092			0.028	.868			3.333	.086			3.634	.057
>60	21	12			24	9			23	10			24	9		
≤60	19	24			32	11			21	22			22	21		
Gender			0.038	.846			0.400	.527			0.015	.902			1.058	.304
Male	18	17			27	8			20	15			19	16		
Female	22	19			29	12			24	17			27	14		
Diameter (cm)			4.736	.030			9.872	.001			4.589	.032			8.873	.003
<1 cm	5	12			7	10			6	11			5	12		
≥1 cm	35	24			49	10			38	21			41	18		
Lymphatic metastasis			5.576	.018			4.877	.027			5.836	.016			4.787	.029
Yes	34	22			45	11			37	19			38	18		
No	6	14			11	9			7	13			8	12		
TNM stage			8.313	.004			7.852	.005			12.048	.001			7.403	.007
I–II	5	15			10	10			5	15			7	13		
III–IV	35	21			46	10			39	17			39	17		
Membrane invasion			5.076	.024			25.542	.001			9.431	.002			4.311	.038
Yes	28	16			42	2			32	12			31	13		
No	12	20			14	18			12	20			15	17		

SP1 = transcription factor1, TC = thyroid cancer.

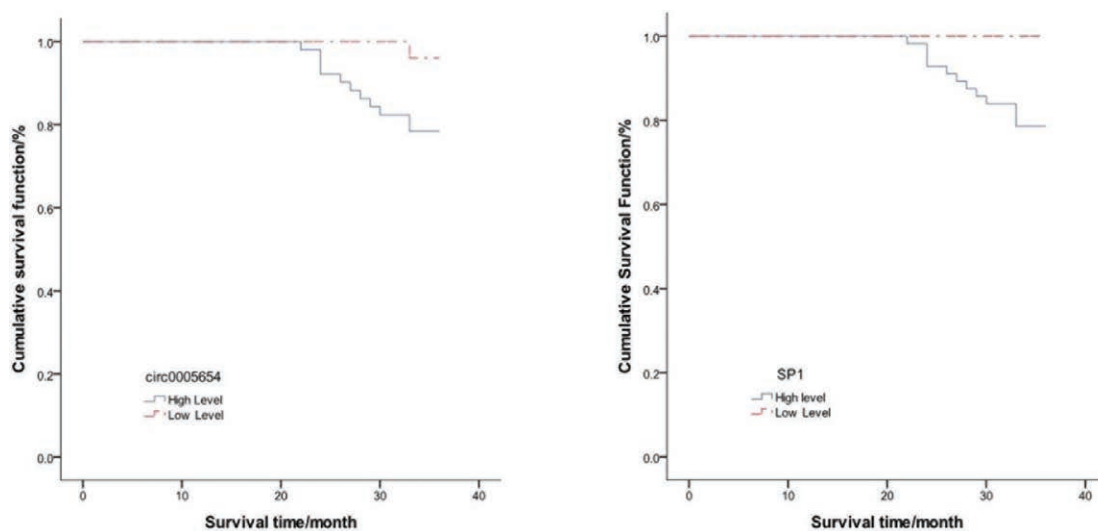


Figure 4. The survival rate of thyroid cancer.

Table 3

Comparison of the survival rates of patients with different levels of Circ0005654 and SP1 expression in thyroid cancer.

Detection index	Cumulative survival rate at 3 yr	χ^2	P
Circ0005654		3.879	.049
High expression	78.4% (40/51)		
Low expression	96.0% (24/25)		
SP1		4.973	.029
Positive	78.6% (44/56)		
Negative	100% (20/20)		

SP1 = transcription factor1.

in thyroid cancer. The expression of Circ0005654 in thyroid cancer and adjacent tissues was determined by sqRT-PCR. We found that expression in cancer tissue was significantly higher than that of adjacent tissues. Moreover, through the ROC analysis, we could

show that the ROC area of Circ0005654 of 0.9553 (95% confidence interval: 0.9211–0.9895), the cutoff value of 0.7895, the sensitivity of 92.11%, and the specificity level of 86.84% were indicative of TC.

It has been shown^[19] that SP1 acts as a transcription factor, can manipulate a signaling pathway for ovarian cancer progression, and has obvious oncogenic effects. Moreover, SP1 expression is aberrant in many cancers and is associated with poor prognosis^[20] such as in thyroid, gastric, pancreatic, lung, and breast cancers.^[21–23] In thyroid cancer tissues, SP1 influences sodium/iodine isotropic transporters. Its expression in thyroid cancer and adjacent tissues was examined by IHC. The results indicate that the positive expression rate of SP1 in thyroid cancer tissues is significantly higher than that of adjacent tissues. The expression of SP1 is closely related to the clinicopathology of thyroid cancer tissue and is related to lymph node metastasis, capsule invasion, tumor diameter, and TNM stage. In addition, it may affect the postoperative survival of the patients. The 3-year-cumulative survival rate of patients displaying a high SP1 expression, was 78.6% lower than that of patients with low SP1 expression, indicating that the SP1 expression level of thyroid cancer patients is closely related to the clinicopathological parameters.

As a classical promoting cancer pathway, the Wnt/ β -catenin signaling pathway plays a role in various malignancies. Previous studies have found that^[14] Circ0005654 interacts with SP1 in GC tissues to promote the invasion and metastasis of gastric cancer cells and to promote gastric cancer progression. Circ0005654 was identified as a miR-363 sponge that enhanced SP1 expression. Moreover, SP1 promotes carcinogenesis in gastric cancer by regulating myc transcription to enhance the Wnt/ β -catenin pathway. Therefore, the present study additionally tested Wnt/ β -catenin expression in thyroid cancer tissues. The results showed that the expression level of Wnt/ β -catenin was significantly higher in cancer tissues, which is consistent with the SP1 expression, therefore, we speculated that Circ0005654 promotes the progression of thyroid cancer through mediating Wnt/ β -catenin expression.

5. Conclusion

Circ0005654 and SP1 were detected in thyroid cancer and found to be related to patient clinicopathological characteristics and prognosis. The mechanism of promoting the progression of thyroid cancer may be related with the activation of the Wnt/ β -catenin signaling pathway. However, this study has some shortcomings. First, this paper is a single-center study and the number of samples is small, which may bias statistics. Secondly, the correlation between Circ0005654 and SP1 expression, determined from patient specimens, needs to be further verified by in vitro and in vivo experiments, which will be the focus of our later work.

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Supervision: Sheng-Jin Han.

Visualization: Sheng-Jin Han.

Writing – original draft: Cui Yang.

Writing – review & editing: Sheng-Jin Han.

References

- [1] Li X, Ding J, Wang X, et al. NUDT21 regulates circRNA cyclization and ceRNA crosstalk in hepatocellular carcinoma. *Oncogene*. 2020;39:891–904.
- [2] Zhang S. The characteristics of circRNA as competing endogenous RNA in pathogenesis of acute myeloid leukemia. *BMC Cancer*. 2021;21:277–86.
- [3] Kristensen LS, Jakobsen T, Hager H, et al. The emerging roles of circRNAs in cancer and oncology. *Nat Rev Clin Oncol*. 2022;19:188–206.
- [4] Zhang Q, Wang W, Zhou Q, et al. Roles of circRNAs in the tumor microenvironment. *Mol Cancer*. 2020;19:14–29.
- [5] Zhou GR, Huang DP, Sun ZF, et al. Characteristics and prognostic significance of circRNA-100876 in patients with colorectal cancer. *Eur Rev Med Pharmacol Sci*. 2020;24:11587–93.
- [6] Zhang Q, Wu L, Liu SZ, et al. Hsa_circ_0023990 promotes tumor growth and glycolysis in dedifferentiated TC via targeting miR-485-5p/FOXO1 axis. *Endocrinology*. 2021;162:bqab172.
- [7] Chen L, Sun Z, Xu H, et al. Emerging roles of circular RNAs in thyroid cancer. *Exp Cell Res*. 2021;404:112626–36.
- [8] Zhu G, Chang X, Kang Y, et al. CircRNA: a novel potential strategy to treat thyroid cancer (Review). *Int J Mol Med*. 2021;48:201–13.
- [9] Xiong H, Yu H, Jia G, et al. circZFR regulates thyroid cancer progression by the miR-16/MAPK1 axis. *Environ Toxicol*. 2021;36:2236–44.
- [10] Shi P, Liu Y, Yang D, et al. CircRNA ZNF609 promotes the growth and metastasis of thyroid cancer in vivo and in vitro by downregulating miR-514a-5p. *Bioengineered*. 2022;13:4372–84.
- [11] Fan YX, Shi HY, Hu YL, et al. Circ_0000144 facilitates the progression of thyroid cancer via the miR-217/AKT3 pathway. *J Gene Med*. 2020;22:e3269.
- [12] Wang T, Huang Y. Downregulation of hsa_circ_0001681 suppresses epithelial-mesenchymal transition in thyroid carcinoma via targeting to miR-942-5p/TWIST1 signaling pathway. *J Bioenerg Biomembr*. 2021;53:609–20.
- [13] Wang Y, Xu S, Chen Y, et al. Identification of hsa_circ_0005654 as a new early biomarker of gastric cancer. *Cancer Biomark*. 2019;26:403–10.
- [14] Yang C, Han S. The circular RNA circ0005654 interacts with specificity protein 1 via microRNA-363 sequestration to promote gastric cancer progression. *Bioengineered*. 2021;12:6305–17.
- [15] Ding W, Zhao S, Shi Y, et al. Positive feedback loop SP1/SNHG1/miR-199a-5p promotes the malignant properties of thyroid cancer. *Biochem Biophys Res Commun*. 2020;522:724–30.
- [16] Wang H, Yan X, Zhang H, et al. CircRNA circ_0067934 overexpression correlates with poor prognosis and promotes thyroid carcinoma progression. *Med Sci Monit*. 2019;25:1342–9.
- [17] Yang W, Bai C, Zhang L, et al. Correlation between serum circRNA and thyroid micropapillary carcinoma with cervical lymph node metastasis. *Medicine (Baltim)*. 2020;99:e23255.
- [18] Guo D, Li F, Zhao X, et al. Circular RNA expression and association with the clinicopathological characteristics in papillary thyroid carcinoma. *Oncol Rep*. 2020;44:519–32.
- [19] Cui PH, Li ZY, Li DH, et al. SP1-induced lncRNA DANCR contributes to proliferation and invasion of ovarian cancer. *Kaohsiung J Med Sci*. 2021;37:371–8.
- [20] Beishline K, Azizkhan-Clifford J. Sp1 and the “hallmarks of cancer”. *FEBS J*. 2015;282:224–58.
- [21] Chiefari E, Brunetti A, Arturi F, et al. Increased expression of AP2 and Sp1 transcription factors in human thyroid tumors: a role in NIS expression regulation? *BMC Cancer*. 2002;2:35–8.
- [22] Wang L, Wei D, Huang S, et al. Transcription factor Sp1 expression is a significant predictor of survival in human gastric cancer. *Clin Cancer Res*. 2003;9:6371–80.
- [23] Jiang NY, Woda BA, Banner BF, et al. Sp1, a new biomarker that identifies a subset of aggressive pancreatic ductal adenocarcinoma. *Cancer Epidemiol Biomarkers Prev*. 2008;17:1648–52.