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Original Article

Correlations of PCNA expression with thyroid cancer ultrasound and histopathologic features

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Abstract: Objective: To investigate the correlations of proliferating cell nuclear antigen (PCNA) gene expression with thyroid cancer (TC) ultrasound (US) features, histopathology and clinical stage. Methods: A total of 66 TC patients admitted and treated in the Department of Oncology of our hospital from April 2014 to April 2018 were enrolled randomly. The conventional US imaging data of the patients were collected. Paired carcinoma and para-carcinoma tissues were obtained after operation to detect the expression of PCNA protein by immunohistochemistry (IHC). The correlations of PCNA expression with the patients' US manifestations and clinical stages were analyzed. Results: The positive rate of PCNA was 72.73% (48/66) in TC tissues and 13.64% (9/66) in paired para-carcinoma tissues, displaying a statistically significant difference between the two groups ($P<0.05$). The PCNA and US features suggested that there was no significant difference in tumor boundary between the PCNA positive group and PCNA negative group ($P>0.05$). However, significant differences in tumor diameter, echo, calcification and blood flow were found between the two groups ($P<0.05$). The pathologic data of preoperative US diagnosis and PCNA expression in postoperative TC specimens were analyzed, and the results indicated that PCNA expression was prominently associated with T stage and N stage in US diagnosis ($P<0.05$). The total correct rate of US in assessing the T stage was 75.8% (50/66), and the over-staging rate and under-staging rate in evaluating the T stage were 13.6% (9/66) and 10.6% (7/66), respectively. Conclusion: The expression of PCNA protein in TC tissues is significantly correlated with the diameter, echo, calcification and blood flow of US features as well as clinical stage detected by US. PCNA level and US examination can provide certain clinical values for TC treatment.

Keywords: Thyroid cancer, PCNA, ultrasound, tumor stage

Introduction

Thyroid cancer (TC) is one of the most common malignant tumors in endocrine organs, and surveys have shown that the incidence rate of thyroid nodules is as high as 50% in people aged over 50 years old [1, 2]. Moreover, about 5% thyroid nodules are malignant. In recent years, the morbidity and mortality rates of TC have been rising, with an annual average growth rate of up to 4% [3]. There are three subtypes of TC, namely, papillary TC (PTC), follicular TC (FTC) and anaplastic TC (ATC), of which FTC possesses the highest morbidity rate (nearly 80%), and ATC is the most aggressive [4, 5]. Studies have manifested that epigenetic alterations may play crucial roles in the occurrence and development of TC.

Proliferating cell nuclear antigen (PCNA) only exists in normal proliferating cells and tumor cells. It is a key protein of abnormal cell proliferation, has a close association with deoxyribonucleic acid (DNA) synthesis in cells and exerts important effects on cell proliferation [6, 7]. The relationship between PCNA and the occurrence and development of tumor has gradually become a research hotspot in recent years. PCNA gene is highly expressed in many tumors, including gastric cancer, liver cancer, colon cancer, breast cancer, bladder cancer and lung cancer [8, 9]. TC often metastasizes to the cervical lymph nodes (LN), so its early discovery is of great importance for the planning, surgery and management of patients. Ultrasound (US) is the preferred imaging method for diagnosing TC. In addition, it can provide guidance for fine-needle aspiration biopsy (FNAB) [10].

In this paper, the data of 66 outpatients and inpatients with TC in our hospital from April

2014 to April 2018 are retrospectively summarized, and the PCNA expression is combined with the US features and clinical stage, so as to explore the clinical application value of US combined with PCNA in detecting TC.

Data and methods

Clinical data

A total of 66 TC patients aged 21.5-75.8 years, with an average of (46.7±15.3) years old (34 males and 32 females), during April 2014 and April 2018 were enrolled into a retrospective study. All the patients received US scanning and cytologic diagnosis via FNAB. There were 44 cases of PTC (66.67%), 12 cases of FTC (18.18%), 3 cases of medullary TC (4.55%) and 7 cases of undifferentiated carcinoma (10.60%). This study was performed according to the principles of the Declaration of Helsinki, and the written informed consent was obtained from all the participants. The study was approved by the Ethics Committee of Jiading District Central Hospital Affiliated Shanghai University of Medicine & Health Sciences.

US examination

Instruments: A high-resolution US system (Aplio ultrasonic machine, Toshiba Medicine, Apt, France) equipped with a 14 MHz high-energy linear probe was utilized. The patients were in the supine and lateral positions to fully expose the anterior region of neck, and a comprehensive scanning in transverse, longitudinal and beveled sections was conducted for two lobes and isthmus of the thyroid gland and bilateral cervical LNs. Two-dimensional grayscale US was applied to carefully observe the location, size, number, boundary, internal echo level, whether calcification or not, etc. of the thyroid goiter. The blood flow in every nodule was graded through color Doppler US imaging and Alder semi-quantitative method. Both sides of the neck were examined carefully, and whether there was swelling of LN was observed and recorded. US diagnostic criteria for LN metastasis: local or diffuse high-level echo, fine or coarse calcification, cystic change and suborbicular shape (ratio of major axis to minor axis <1.5).

Pathologic examination via hematoxylin and eosin (H&E) staining

A total of 66 cases of TC biopsy specimens were fixed in 4% formaldehyde, then routinely dehydrated and embedded in paraffin blocks. Later, the paraffin blocks were sliced to 4-μm-thick sections, followed by H&E staining and histopathological observation.

PCNA protein level detected via immunohistochemistry (IHC)

The 66 cases of TC tissues excised through surgery and paraffin blocks of paired para-carcinoma tissues were sliced (4-μm-thick) from the tumor center in the axial plane, so as to guarantee the correlation with preoperative US. PCNA antibody was purchased from Abcam (article number: Ab8197, Cambridge, the UK). IHC kit was bought from Zhongshan Jinqiao Agent (Guangzhou, China). All the experimental operations were carried out in accordance with the instructions provided.

The IHC results were reviewed and scored by one experienced pathologist. The IHC score was calculated according to the percentage and staining intensity of positive cells under the field of vision: 0 points (no positive cells), 1 point (positive cells percentage =1-10%), 2 points (positive cells percentage =11-50%), 3 points (positive cells percentage =51-80%) and 4 points (positive cells percentage =81-100%). Staining intensity of positive cells: negative =0 points, weakly positive =1 point, moderately positive =2 points and strongly positive =3 points. The product of the two scores was regarded as the IHC score of the lesion. The total IHC score was 0-12 points, of which 0-1 point was defined as negative expression, and 2-12 points as positive expression.

Statistical methods

The experimental results were analyzed using statistical software GraphPad Prism (Version 5.01, GraphPad Company, Santiago de Chile). Chi-square test was adopted to analyze the difference in PCNA expression betweencolorectal carcinoma tissues and para-carcinoma tissues and compare the correlations of PCNA expression with US parameters. $P<0.05$ was considered significant.

Results

Detection of TC histopathology and PCNA protein expression

All the 66 patients were definitely diagnosed with TC through biopsy or pathologic examina-

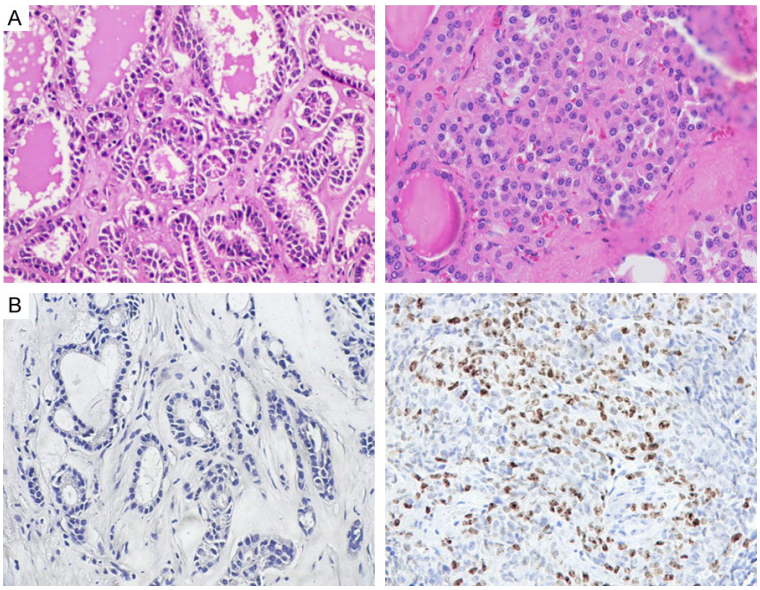

Figure 1. Detection of TC histopathology and PCNA protein expression. A: Histologic appearance of TC tissues and para-carcinoma tissues examined by H&E staining. B: PCNA protein expression detected by IHC (×400).

Table 1. Difference in PCNA protein expression between TC tissues and para-carcinoma tissues

Group	N	PCNA	
		Positive	Negative
TC tissue	66	48 (72.73%)	18 (27.27%)
Para-carcinoma tissue	66	9 (13.64%)	57 (86.36%)
X^2		8.25	
P		0.0081	

tion after surgical resection. There were 44 cases of PTC (66.67%), 12 cases of FTC (18.18%), 3 cases of medullary TC (4.55%) and 7 cases of undifferentiated carcinoma (10.60%) (**Figure 1A**). Furthermore, PCNA protein expressions in TC tissues and para-carcinoma tissues were measured by virtue of the IHC semi-quantitative method. According to **Table 1**, PCNA was located in the nucleus, and the positive rate of PCNA was 72.73% (48/66) in TC tissues and 13.64% (9/66) in paired para-carcinoma tissues, displaying a statistically significant difference ($P<0.05$).

US image data

Solitary nodules or masses in the tumor body were visible under US, with irregular and lobulated shape. Different degrees of low-density regions could be seen in the focus, showing blurred boundary, unclear division with surrounding fat spaces and organs and calcification in most cases (**Figure 2**).

Relationship between PCNA protein expression in TC tissues and US features

The US images and pathologic image samples of IHC staining of all the enrolled patients were analyzed. It could be seen that there was no significant difference in tumor boundary between PCNA positive group and PCNA negative group ($P>0.05$). However, significant differences in tumor diameter, echo, calcification and blood flow were discovered between the two groups ($P<0.05$) (**Table 2**).

Relationship between US stage and PCNA expression

The pathologic data of US diagnosis before operation and PCNA expression in postoperative TC specimens were analyzed, and the results indicated that in US diagnosis, there were 19 cases of T1, 17 cases of T2, 20 cases of T3 and 10 cases of T4. Moreover, PCNA expression had prominent correlations with T stage and N stage in US diagnosis ($P<0.05$) (**Table 3**).

Relationship between US stage and pathologic staging examination

The total correct rate of US in assessing the T stage was 75.8% (50/66), and the over-staging rate and under-staging rate in evaluating the T-stage were 13.6% (9/66) and 10.6% (7/66), respectively (**Table 4**).

Discussion

As a heterogeneous disease, TC is characterized by initiation of gene mutation in signaling pathways and abnormalities of suppressor genes and cyclins [11]. PCNA gene possesses a special loop structure and serves as a core component of DNA replication complex. In addi-

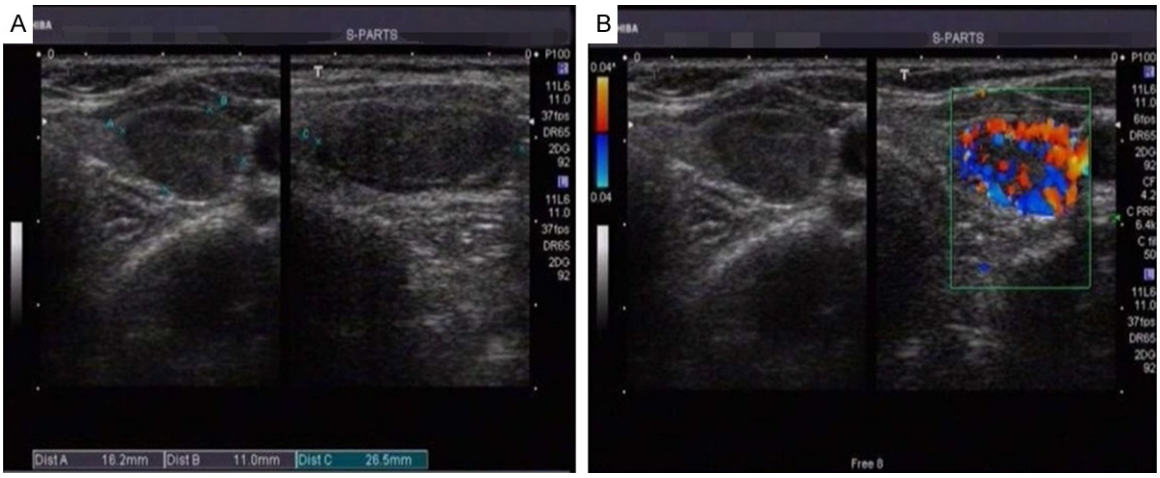

Figure 2. US image data of TC patients. A male patient aged 54 years old. A: An approximately 0.9×0.4 cm hypoechoic nodule is detected at the position near the isthmus of right thyroid gland, the internal echo is not homogenous, and the boundary is clear and regular. B: Fairly abundant blood flow signals are visible in color Doppler flow image (CDFI), dominated by the peripheral regions and displaying encircled features. The internal echo of the thyroid parenchyma around the nodule is homogeneous.

Table 2. Relationship between US features and PCNA protein expression

US	N (66)	PCNA expression		χ^2	
		Positive (48)	Negative (18)		
Diameter					
≤5 mm	32	20	12	3.52	<0.05
>5 mm	34	28	6		
Echo					
Low-level echo	29	20	9	3.24	<0.05
Equal echo	22	16	6		
Mixed echo	15	12	3		
Boundary					
Clear	32	25	7	0.91	>0.05
Blurred	34	23	11		
Calcification					
Yes	26	23	3	5.35	<0.05
No	40	25	15		
Blood flow					
I-II	39	29	10	4.51	<0.05
III-IV	27	19	8		

tion, it participates in multiple vital functions such as cell cycle regulation, DNA damage repair, DNA methylation and chromosome remodeling by binding to various intracellular molecules [12]. Also, PCNA is closely related to cell apoptosis and proliferation. The abnormal expression of PCNA has a close association with the occurrence and development of tumors, so it can be applied to assess the malignancy and proliferation potential of the tumors, and its expression level varies in different stages of the tumors [13]. In this study, 66 TC patients were enrolled to determine the expression level of PCNA protein in TC tissues and para-carcinoma tissues by means of the IHC. Consistent with the study of Inoue et al [14, 15], it was revealed in this study that the positive rate of PCNA in TC tissues [72.73% (48/66)] was remarkably higher than that in paired para-carcinoma tissues [13.64% (9/66)] (P<0.05). High-resolution US can detect individual thyroid nodule with a high frequency in 19-67% females and elderly people, while the importance of detecting the thyroid nodules in clinic is to exclude the possibility of TC. Furthermore, the US-guided NAB is also a major tool for diagnosing cervical metastasis of TC. An amount of literature has reported the US features of TC tissues, including calcification, cystic change, echoic loss of fat mass, high echo, round shape and vascular abnormality on US image, while metastatic LNs are prone to manifesting large round shape, low-level echo, high vascularization and loss of gate structure. In DTC, metastatic LNs may also show particular characteristics, such as high echo points or microcalcification and cystic

Table 3. Relationship between US staging and PCNA expression

US diagnosis		N (66)	PCNA expression in tumor body		χ^2	*P*
			Positive (48)	Negative (18)		
T stage	T1	19	5	14	7.54	0.012
	T2	17	7	10		
	T3	20	12	8		
	T4	10	8			
N stage	N0	31	20	11	5.14	0.013
	N1	16	10	6		
	N2	19	16	3		

Table 4. Comparisons of results of MRI staging and diagnosis and postoperative pathological T stage (n)

Pathological stage	N	US stage				
		T1	T2	T3	T4	Accuracy rate
T1	21	16	5	0	0	76.2%
T2	15	2	11	2	0	73.3%
T3	19	1	1	15	2	78.9%
T4	11	0	0	3	8	72.7%
Total	66	19	17	20	10	75.8%

manifestations [16]. Latest investigations have demonstrated the excellent diagnostic performance of the combination of various US features. However, there are few retrospective studies connecting PCNA expression in TC tissues and US features to the patients' clinical stage, so as to realize systematic evaluation.

According to a majority of reports, it is discovered by chance that TC follows a slow process, and US proves that irregular or blurry nodule edge and vascular pattern and microcalcifications inside the nodule are closely associated with malignant changes of the tumor [17]. In current study, the US and clinical and pathological data of 66 TC patients were retrospectively investigated. It was found that the tumor region of TC mostly displays hypoechoic nodules, and the LN metastasis shows punctate microcalcifications which are stratified, calcified and spherical in cytology. On the other hand, low-level or equal echo may also serve as an important detection factor for TC. CDFI can macroscopically evaluate the blood flow signal in TC tumor before operation [18]. In this study, there were few blood flow signals in normal thyroid tissues, but abundant blood flow signals were visible in TC masses, which were in scattered strip and branch shapes. It is reported that the thrombus calcification of tumor can imply the diagnosis of malignant tumor to a great extent. In this study, the number of calcification in PCNA positive group was greater than that in PCNA negative group.

Furthermore, the results of combining with PCNA indicated that there were no significant differences in tumor boundary and calcification between PCNA positive group and PCNA negative group ($P>0.05$), but significant differences in tumor diameter, echo and blood flow existed between the two groups ($P<0.05$). PCNA is a cell proliferation-associated indicator, and it was shown in this study that the tumor diameter was increased markedly in PCNA positive group compared with that in PCNA negative group, which is consistent with the tendency. Since the tumor in PCNA positive group grew vigorously, possessing relatively rich angiogenesis and blood supply, strong blood flow signals of positive PCNA were visible on CDFI. According to the clinical staging results through US diagnosis, there were 19 cases of T1, 17 cases of T2, 20 cases of T3 and 10 cases of T4. The total correct rate of US in assessing the T stage was 75.8% (50/66), and the over-staging rate and under-staging rate in evaluating the T stage were 13.6% (9/66) and 10.6% (7/66), respectively, which are in agreement with the findings of Yokozawa T et al [19, 20], suggesting that US has certain values in diagnosing the clinical stage of TC. Moreover, the pathologic data of US diagnosis before operation and PCNA expression in postoperative TC specimens were analyzed, and PCNA expression had prominent correlations with T stage and N stage in US diagnosis.

Conclusion

In conclusion, the expression of PCNA protein in postoperative TC tissues is significantly correlated with the diameter, echo, calcification, and blood flow of US features as well as the clinical stage detected by US. The PCNA level and US examination can provide certain clinical values for TC treatment and monitoring.

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Disclosure of conflict of interest

None.

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