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Correlation of thyroid papillary carcinoma CEUS characteristics with cancer cell proliferation and invasion

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ABSTRACT

Objective: To study the correlation of thyroid papillary carcinoma CEUS characteristics with cancer cell proliferation and invasion. **Methods:** A total of 128 patients with thyroid papillary carcinoma who received surgical treatment in the hospital between May 2013 and May 2016 were collected, CEUS was used to make clear the peak intensity (PI) and area under the curve (AUC) of tumor tissue and surrounding normal tissue, and the median of PI and AUC was referred to further divide the patients into high PI group and low PI group as well as high AUC group and low AUC group, 64 cases in each group. Fluorescent quantitative PCR was used to determine proliferation and invasion gene mRNA expression in tumor tissues. **Results:** PI and AUC levels in tumor tissue were lower than those in surrounding normal tissue; proliferation genes EZH2, Livin, hTERT, HMGA1 and Wip1 mRNA expression of low PI group were higher than those of high PI group, and invasion gene Ki-67 mRNA expression was higher than that of high PI group while P53 and MRP-1 mRNA expression were lower than those of high PI group; proliferation genes EZH2, Livin, hTERT, HMGA1 and Wip1 mRNA expression of low AUC group were higher than those of high AUC group, and invasion gene Ki-67 mRNA expression was higher than that of high AUC group while P53 and MRP-1 mRNA expression were lower than those of high AUC group. **Conclusion:** Thyroid papillary carcinoma CEUS parameters PI and AUC levels can quantifiably reflect the cancer cell proliferation and invasion activity.

1. Introduction

Thyroid papillary carcinoma is common in clinical practice, its malignant degree is low, but after detected, it should also be treated early to avoid the increased malignant degree and metastasis of tumor[1]. Ultrasound is one of the most reliable means for thyroid disease screening, but the sensitivity and specificity of the conventional two-dimensional screening are lower, and the clinical missed diagnosis rate is high. Contrast-enhanced ultrasonography (CEUS) uses contrast agents to show the microvessels in lesions, and indirectly judges the lesion properties by perfusion, and it has already been popular in the diagnosis and the differentiation of thyroid diseases[2,3]. In the study, the CEUS was used for thyroid papillary carcinoma inspection, and the correlation of thyroid papillary carcinoma CEUS characteristics with cancer cell proliferation and invasion was specifically analyzed.

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2. Subjects and methods

2.1. Inclusion and exclusion criteria

Inclusion criteria: (1) diagnosed with thyroid papillary carcinoma by thyroid tissue biopsy; (2) diagnosed for the first time and without previous history of thyroid tumor treatment; (3) with primary tumor. Exclusion criteria: (1) associated with malignant tumor diseases of other tissue organs; (2) pregnant or breastfeeding women; (3) not cooperating with related examination, and with clinical data missing.

2.2 Case information

A total of 128 patients with thyroid papillary carcinoma who received surgical treatment in the hospital between May 2013 and May 2016 were selected as the research subjects, and the patients themselves signed the informed consent. They included 68 male cases and 60 female cases that were 26-69 years old. The study was approved by hospital ethics committee.

2.3 CEUS

After admission (before the operation), the color Doppler diasonograph (Xuzhou Kaixin Electronic Instrument Co., Ltd, the article number DCU10) was used for contrast-enhanced ultrasonography, contrast agent was Bracco SonoVue and added in 5 mL saline in advance to configure microbubble suspension. For ultrasonography, 2.4 mL was injected via the cubital superficial vein, and then 5 mL saline was injected to flush tube. Before ultrasonography, conventional two-dimensional ultrasound was conducted at first, thyroid observation section was selected, patients were instructed not to swallow and the mode was switched to the ultrasonography, the dynamic storage key was pressed at the same time of contrast agent injection, and the scanning lasted for 2 min. Later, the software was used to sketch the region of interest (ROI) of thyroid lesions and surrounding normal tissues, and draw time-intensity curve (TIC), and the peak intensity (PI) and the area under the curve (AUC) were quantitatively analyzed.

2.4 Cancer cell proliferation and invasion gene mRNA expression

Thyroid cancer tissue samples from radical operation for thyroid cancer were taken, added in Trizol reagent (Shanghai Genmed Genetic Pharmaceutical Technology Co., LTD., the article number GMS12279) and 0.2 mL chloroform (Wuhan Khayal Bio-technology Co., LTD., the article number 0219400225) and centrifuged at low temperature (4 ℃) and 12 000 rpm for 15 min to get supernatant and add it into same volume of isopropyl alcohol (Nanjing Mr Ng Biological Technology Co., LTD., the article number 0918-1L) for form gel block (total RNA). The gel block was added in 75% ethanol (Shanghai Luwen Bio-technology Co., LTD., the article number LWJSKJ025-608), washed and then dried at room temperature for 5-10 min. Ultraviolet absorption spectrometry was used to detect RNA purity and concentration, reverse transcription kits (Biomiga China, article number RT021301/02/03) were used to synthesize sample cDNA, fluorescence quantitative PCR kits (Shenzhen Zike Biological Science and Technology Co., LTD., article number zk7340) were used for mRNA amplification of proliferation genes EZH2, Livin, hTERT, HMGA1 and Wip1 as well as invasion genes Ki-67, P53 and MRP-1. Amplification curve was obtained in computer software, and target gene mRNA expression was calculated.

2.5 Statistical methods

Data obtained in the study was calculated by personnel with professional statistical knowledge, measurement data was in terms of (mean ± SD) and comparison between groups was by grouping t test. $P<0.05$ was set as the standard of statistical significance in difference.

3. Results

3.1 CEUS parameters

Comparison of CEUS parameters PI (dB) and AUC (dB s) in tumor tissue and surrounding normal tissue of patients with thyroid papillary carcinoma was as follows: PI and AUC levels in tumor tissue were significantly lower than those in surrounding normal tissue, and differences were statistically significant ($P<0.05$), shown in Table 1. PI median of tumor tissue was 11.94 and AUC median was 1793.62, and they were used as boundary to divide the patients into high PI group and low PI group as well as high AUC group and low AUC group, 64 cases in each group.

Table 1.

Comparison of CEUS parameter levels between two groups of thyroid tumor tissues.

Groups	*n*	PI	AUC
Tumor tissue	128	11.26±1.94	1 832.48±200.74
Surrounding normal tissue	128	13.52±2.41	2 093.61±243.81
T value		7.393	15.931
P value		<0.05	<0.05

3.2 Tumor tissue PI value and proliferation gene mRNA expression

Comparison of proliferation genes EZH2, Livin, hTERT, HMGA1 and Wip1 mRNA expression in thyroid papillary carcinoma tissue between high PI group and low PI group was as follows: EZH2, Livin, hTERT, HMGA1 and Wip1 mRNA expression in tumor tissue of low PI group were significantly higher than those of high PI group, and differences in EZH2, Livin, hTERT, HMGA1 and Wip1 mRNA expression in thyroid papillary carcinoma tissue were statistically significant between high PI group and low PI group ($P<0.05$), shown in Table 2.

Table 2.

Tumor tissue PI value and proliferation gene mRNA expression.

Groups	*n*	EZH2	Livin	hTERT	HMGA1	Wip1
High PI group	64	130.64±15.88	139.72±15.88	98.36±11.58	142.43±18.66	129.38±15.92
Low PI group	64	173.28±23.19	180.25±21.64	168.35±19.22	192.17±25.38	184.36±22.47
T value		12.846	18.624	15.482	17.148	19.279
P value		<0.05	<0.05	<0.05	<0.05	<0.05

3.3 Tumor tissue PI value and invasion gene mRNA expression

Comparison of invasion genes Ki-67, P53 and MRP-1 mRNA expression in thyroid papillary carcinoma tissue between high PI group and low PI group was as follows: Ki-67 mRNA expression in tumor tissue of low PI group was significantly higher than that of high PI group while P53 and MRP-1 mRNA expression were significantly lower than those of high PI group, and differences in Ki-67, P53 and MRP-1 mRNA expression in thyroid papillary carcinoma tissue were statistically significant between high PI group and low PI group ($P<0.05$), shown in Table 3.

Table 3.

Tumor tissue PI value and invasion gene mRNA expression.

Groups	*n*	Ki-67	P53	MRP-1
High PI group	64	99.23±10.54	112.46±15.39	131.17±15.38
Low PI group	64	164.38±20.94	74.39±9.11	83.29±10.17
T value		16.93	10.73	15.36
P value		<0.05	<0.05	<0.05

3.4 Tumor tissue AUC value and proliferation gene mRNA expression

Comparison of proliferation genes EZH2, Livin, hTERT, HMGA1 and Wip1 mRNA expression in thyroid papillary carcinoma tissue between high AUC group and low AUC group was as follows: EZH2, Livin, hTERT, HMGA1 and Wip1 mRNA expression in tumor tissue of low AUC group were higher than those of high AUC group, and differences in EZH2, Livin, hTERT, HMGA1 and Wip1 mRNA expression were statistically significant between high AUC group and low AUC group ($P<0.05$), shown in Table 4.

3.5 Tumor tissue AUC value and invasion gene mRNA expression

Comparison of invasion genes Ki-67, P53 and MRP-1 mRNA expression in thyroid papillary carcinoma tissue between high AUC group and low AUC group was as follows: Ki-67 mRNA expression in tumor tissue of low AUC group was higher than that of high AUC group while P53 and MRP-1 mRNA expression were lower than those of high AUC group, and differences in Ki-67, P53 and MRP-1 mRNA expression were statistically significant between high AUC group and low AUC group ($P<0.05$), shown in Table 5.

Table 5.

Tumor tissue AUC value and invasion gene mRNA expression.

Groups	*n*	Ki-67	P53	MRP-1
High AUC group	64	98.73±11.52	125.49±15.63	149.37±18.52
Low AUC group	64	143.28±16.93	90.37±9.82	94.82±10.18
T value		16.488	9.239	14.384
P value		<0.05	<0.05	<0.05

4. Discussion

CEUS is the technology that uses contrast agents to enhance backscattering echo and improve ultrasound diagnosis resolution and specificity, and with the ultrasonic instrument innovation, the technology has been widely used in screening and differential diagnosis of heart, liver, kidney, brain and other important organ diseases[4,5]. Thyroid papillary carcinoma accounts for more than 85% of all malignant thyroid diseases, the age of onset trends to be younger, its malignant degree is low, but it can still spread from the primary site to other nodes through the lymph vessels in gland, and early diagnosis of disease and judgment of its malignant degree can provide important basis for the implementation of the clinical treatment. In the study, CEUS was used for inspection of thyroid papillary carcinoma, and it was found that PI and AUC levels in tumor tissue were lower than those in surrounding normal tissue. The CEUS of normal thyroid tissue is quick homogeneous coherence-enhancing, the normal vascular structure and perfusion status change after thyroid nodule formation, the central vascular distribution is relatively small after thyroid papillary carcinoma formation, tumor embolus, fibrosis and others all increase vascular ischemia, these factors cause that the CEUS enhanced mode is mostly characterized by low enhancement, and lead to above research results.

CEUS PI and AUC levels can be used to judge the character of thyroid tissue, but it is less covered at present whether the indicators can quantitatively measure the malignant degree of thyroid papillary carcinoma. In the study, the median of PI and AUC were referred to further divide the thyroid papillary carcinoma patients into high PI group and low PI group as well as high AUC group and low AUC group so as to judge the different tumor malignancy in patients with different parameter levels. Infinite proliferation is the first feature of malignant tumor cells, and it comes from the abnormal expression of a large number of proliferation-related genes[6,7]. It has been reported in different studies that EZH2, Livin, hTERT, HMGA1 and Wip1 are associated with thyroid papillary cancer cell proliferation, the positive expression rate of EZH2 in thyroid cancer tissue is higher than that in thyroid adenoma tissue, Livin gene silencing can inhibit the thyroid cancer cell proliferation and invasion[8], high hTERT expression is closely related to the high proliferation potential of thyroid cancer cells, thyroid cancer cell proliferation activity decreases after HMGA1 gene silencing, and Wip1 gene overexpression directly participates in thyroid cancer cell proliferation[9–11]. In the study, above proliferation gene

Table 4.

Tumor tissue AUC value and proliferation gene mRNA expression.

Groups	*n*	EZH2	Livin	hTERT	HMGA1	Wip1
High AUC group	64	134.27±15.88	110.17±15.48	128.74±15.39	131.66±15.87	130.74±15.92
Low AUC group	64	184.39±21.39	159.73±18.51	176.99±21.42	189.63±23.41	174.37±20.66
T value		16.925	14.022	17.453	18.291	15.424
P value		<0.05	<0.05	<0.05	<0.05	<0.05

mRNA expression levels were compared between thyroid papillary carcinoma patients with different PI and AUC levels, and it was found that proliferation genes EZH2, Livin, hTERT, HMGA1 and Wip1 mRNA expression of low PI group were higher than those of high PI group; proliferation genes EZH2, Livin, hTERT, HMGA1 and Wip1 mRNA expression of low AUC group were higher than those of high AUC group. It shows that papillary thyroid cancer cell proliferation activity is negatively correlated with the CEUS parameters PI and AUC levels.

Cancer cell invasion activity is also an important biological feature to determine the malignant degree of tumor, and detecting invasion-related gene expression can accurately assess tumor shape and predict treatment outcomes[12]. Ki-67 gene mutation is closely related to the invasive change of thyroid papillary carcinoma, and its expression in tumor tissue gradually increases as the tumor invasiveness increases[13]. P53 gene is an important tumor suppressor gene in human body, its function is to maintain normal cell growth, start the programmed cell death and inhibit malignant proliferation of tumor, and tumor cells may undergo malignant transformation in the case of Ki-67 gene expression deletion[14]. Research has confirmed that the positive expression rate of MRP-1 protein in thyroid carcinoma tissue is lower than that in normal thyroid tissue, and its expression deletion can lead to the weakening of intercellular adhesion function and the enhancement of cell invasion function[15,16]. It was found in the study that invasion gene Ki-67 mRNA expression of low PI group was higher than that of high PI group while P53 and MRP-1 mRNA expression were lower than those of high PI group, and invasion gene Ki-67 mRNA expression of low AUC group was higher than that of high AUC group while P53 and MRP-1 mRNA expression were lower than those of high AUC group. It indicates that papillary thyroid cancer cell activity is negatively correlated with CEUS parameters PI and AUC levels.

Thyroid papillary carcinoma CEUS parameters PI and AUC levels are low, and the specific PI and AUC values are negatively correlated with cancer cell proliferation and invasion activity. Contrast-enhanced ultrasonography can early identify thyroid papillary carcinoma and objectively assess its malignant degree, and it is expected to become the reliable means for disease treatment and prognosis judgment.

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