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ORIGINAL ARTICLE

Approach the Invasive Potential with Hurthle Cell Tumors of Thyroid

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Abstract
To observe the expression of P53, CyclinD1, Ki-67, Galectin-3, COX-2, Bcl-2 and approach their contribution on assessing the invasive potential for Hurthle cell tumors. Seventy-three cases of Hurthle cell tumor were collected for immunohistochemistry staining. The patients were followed up with 8 months to 5 years. Tumors were divided into four grades according to invasion and diameter:(1) extremely low risk (27 cases that less than 2 cm and without invasion), (2) low risk (18 cases that within 2–3.9 cm and without invasion), (3) moderate risk (21 cases that achieve 4 cm and without invasion), (4) high risk (7 cases that with invasion of capsule/vessel in spite of the diameter). Immunostaining presented that all 73 cases were positive with Galectin-3, COX-2 and Bcl-2. For each group, P53 positive were 29.6%, 55.6%, 90.5%, 100.0%; CyclinD1 stained with 7.4%,22.2%,52.4%,100.0% and Ki-67 were 0.0%,5.6%,9.5%,28.6%, respectively. The higher risk of tumor, the more cases that positive expressed P53 and CyclinD1. After following up within 49 patients, two of the recurring cases were positive with P53 and CyclinD1 and one of which was also highly expressed Ki-67. Detecting P53, CyclinD1 and Ki-67 might provide reference for invasive potential assessment with Hurthle cell tumors but not Galectin-3, COX-2 and Bcl-2.

Keywords Hurthle cell tumors · Immunohistochemistry · P53 · CyclinD1 · Ki-67

Introduction

Hurthle cell tumor is defined as either completely or mainly (>75%) composed of follicular cells with eosinophilic characteristics, while the concentration of eosinophilic cells without envelope should be ruled out [1]. It was put forward as a separate category by WHO in 2017 because their histological and molecular characteristics were different from follicular neoplasm. In addition, Hurthle cell tumors displayed more lymph node metastasis and poor prognosis than follicular tumors [2–4].

Hurthle cell tumors were classified as adenoma and carcinoma according to whether invading with vascular/capsule or distant metastasis. Though some tumors were diagnosed as adenoma, they still had malignant behavior in the future [5–8]. Galectin-3, COX-2, Bcl-2, CyclinD1, P53 and Ki-67 were reported of highly expression with malignant behavior. We examined their staining in hope of providing a reference for invasive potential assessment with Hurthle cell tumor.

Materials and Methods

Specimen Collection

Seventy-three cases of Hurthle cell tumor with paraffin-embedded tissues were collected from archives of pathology with the first people's hospital and the second people's hospital of Jingmen since 2012 to 2017. The patients were followed up by telephone.

Immunohistochemistry Staining

Antibodies were purchased from Fuzhou maixin biotechnology co. LTD. Immunostaining was based on a 3um thickness slice using the EnVision two-step method. Besides, EDTA (PH = 8.0) was employed for antigen repairing, and DAB was applied for staining with antibody.

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Result Interpretation

All results of immunostaining were agreed by two senior pathologists. More than 25% of the stained tumor cells with random intensity in cytoplasm were considered positive for Galectin-3, COX-2 and Bcl-2. For P53, CyclinD1 and Ki-67, five most positive areas were counted to 200 and the average of staining cells with nucleus was calculated as positive cell index. We accepted the following threshold: positive cell index with P53 reached 20%, CyclinD1 arrived 10%, and Ki-67 got to 5%.

Statistical Analyze

All results of immunostaining were determined positive and negative. A probability of $p < 0.05$ was considered statistically significant by SPSS17.0 software package.

Results

General Information

Seventy-three patients with Hurthle cell tumor were 25–80 years old with a median age of 47. Tumors with infiltration of enveloped and/or vascular is received as malignant, therefore Hurthle cell carcinomas were separated out firstly. Besides, Sippel RS and his research team claimed that [5] Hurthle cell tumors with less than 2 cm should be treated as benign and with more than 4 cm might have malignant potential. Samples in study were divided into four grades: (1) extremely low risk (27 cases that less than 2 cm and without invasion), (2) low risk (18 cases that within 2–3.9 cm and without invasion), (3) moderate risk (21 cases that achieve 4 cm and without invasion), (4) high risk (7 cases that with invasion of capsule/vessel in spite of the diameter).

Immunostaining Results

It is a pity that all markers of Galectin-3, COX-2 and Bcl-2 have no difference between several groups. Beyond our expectation, the three antibodies were colored in different area and strength with each case.

As shown in Table 1, 29.6% with extremely low risk and 55.6% in low risk indicated positive staining of P53. On contrary, most cases with moderate (90.5%) and high risk (100%) were displayed positive with P53. It was up-regulated with high risk group while compared to low and extremely low risk (Table 2). We arrived a feature that the higher risk of tumor, the more cases that positive expressing P53 ($r = 0.561$, $p < 0.001$).

Similarly, CyclinD1 also shown an increasing relation with tumor risk ($r = 0.569$, $p < 0.001$). CyclinD1 expressing in each group was 2/27, 4/18, 11/21 and 7/7, respectively. The differences were significant performed between extremely low risk and moderate/high risk, low risk and high risk (displayed in Table 2).

Table 1 Expression of P53, CyclinD1, Ki-67 in Hurthle cell tumors

Group	Case	P53		CyclinD1		Ki-67	
		+	Ratio	+	Ratio	+	Ratio
Grade 1	27	8	29.6%	2	7.4%	0	0.0%
Grade 2	18	10	55.6%	4	22.2%	1	5.6%
Grade 3	21	19	90.5%	11	52.4%	2	9.5%
Grade 4	7	7	100.0%	7	100.0%	2	28.6%

Grade 1 Extremely low risk, *Grade 2* Low risk, *Grade 3* moderate risk, *Grade 4* high risk

There were 0, 1, 2, 2 cases that high expressed Ki-67 in each group respectively. It seemed that Ki-67 with high risk was up-regulated than extremely low risk ($X2 = 8.196$, $p = 0.004$). Besides, all the five cases which with high index of Ki-67 were also displayed positive of P53 and CyclinD1; In addition, all samples that highly expressing CyclinD1 were positive stained with P53.

Following up

Following up was put into practice with 49 patients. There were 17, 14, 13 and 5 cases in each group. After operations within 8 months to 5 years, there wasn't a patient die with Hurthle cell tumor. Total thyroidectomy was operated upon 7 patients who suffered Hurthle cell carcinoma. Five of them in followed were survived with tumor free after lymph nodes resection partly. A patient took place recurrence with a big tumor of 7.5 cm and the tumor was broken up by operation. Another patient who with a 6 cm adenoma and only accepted tumor resection was suffered regeneration in thyroid. The two samples were positive expressed P53 and CyclinD1, and the second still showed high index of Ki-67.

Table 2 Comparison of p53, CyclinD1 and Ki-67 in groups

Group	P53		CyclinD1		Ki-67	
	X2	*p*	X2	*P*	X2	*p*
Grade 1 and 2	3.025	0.082	2.05	0.152	1.534	0.215
Grade 1 and 3	17.771	<0.001	12.098	0.001	2.683	0.101
Grade 1 and 4	11.165	0.001	24.486	<0.001	8.196	0.004
Grade 2 and 3	4.451	0.035	3.725	0.054	0.215	0.643
Grade 2 and 4	3.5	0.061	12.374	<0.001	2.528	0.112
Grade 3 and 4	0.718	0.379	2.185	0.123	1.556	0.212

Grade 1 Extremely low risk, *Grade 2* Low risk, *Grade 3* moderate risk, *Grade 4* high risk

Discussions

Hurthle cell tumor was determined as malignant from benign depending on invasion or metastasis. It's reported that some patients had a pathological diagnosis of Hurthle cell adenoma after the first tumor resection, but they were recurred later and could be accompanied with malignant [5–8]. In this sense, some scholars suggested that all Hurthle cell tumors should be treated as malignant for total thyroidectomy [9, 10]. But most of Hurthle cell adenoma put up benign behavior. Patients who undergo total thyroidectomy might be exposed to high risk of surgery and required taking replacement with thyroxine in his rest life.

How to evaluate the invasion potential of Hurthle cell tumor? Compared with Hurthle cell carcinoma, Anila KR [11] believed that adenomas with increased cellularity, non-macro follicular architecture, more than 90% of Hurthle cells, absence of background colloid and absence of chronic inflammation might be malignant. Others suggested that tumor size was an important reference for prediction. Krhin B et al. [12] considered that the tumor with a diameter of more than 3 cm had potential of recurrence; Sippel RS proposed [5] that tumors above 4 cm should be treated as malignant, while below 2 cm could be regarded as benign; Zhang YW [13] put forward that Hurthle cell adenomas over 5 cm in diameter have malignant potential. Referring to diameter and invasion, samples in study were divided into four grades: extremely low risk (adenoma that less than 2 cm), low risk (adenoma that within 2–3.9 cm), moderate risk (adenoma that achieve 4 cm), high risk (carcinoma with invasion regardless of diameter).

Hurthle cell tumors mainly contain increased mitochondrion under an electron microscope and rare other organelles. Besides, the increasing of mitochondrial function protein [14], the down-regulated mitochondrial miRNA [15] and pigment protein [16], as well as the abnormal antioxidant gene GPX1 [10] and widespread mitochondrial DNA alterations [17] were reported with Hurthle cell carcinoma. All these studies were limited in laboratory because of highly demands for experiment.

COX-2 was considered to help judging a benign thyroid tumor from malignant [18]; others suggested that COX-2 was also upregulated in thyroiditis and benign nodules, which was less associated with progression [19]. Galectin-3 was reported to serve as a marker in differential diagnosis containing Hurthle cell adenomas and carcinomas [20]; On the contrary, Saleh HA [21] observed that Galectin-3 was also stained in benign lesions and normal thyroid tissue. Additionally, Bcl-2 was questioned too [1]. In this sense, all evidence of COX-2, Galectin-3 and Bcl-2 were controversial. This paradoxical finding could be explained by disequilibrium of pro- and anti-apotosis in normal thyroid tissue. Bcl-2 is increased inducing by prostaglandin E2 that catalyzed from COX-2 in thyroid papillary carcinoma [22]. Both the Galectin-3 and Bcl-2 family have a same C-end sequence and might take a similar anti-apoptotic signaling pathways. According to immunohistochemical staining in this study, though different strength and area were colored, all 73 cases of Hurthle cell tumors were interpreted positive expression with COX-2, Galectin-3 and Bcl-2. We considered that the three markers might work by a similar signaling pathway and play a light role in differentiating benign Hurthle cell tumors from malignant.

P53, CyclinD1 and Ki-67 were evidences for cell proliferation and accepted as unfavourable prognosis for numerous tumors. For example, over-expression of P53 was considered as a forecast for recurrence of ovarian cancer [23]; CyclinD1 might promote and encourage a gastric cardia adenocarcinoma [24]; high index of Ki-67 was a marker for regenerating of non-muscle-invasive bladder cancer [25]. High index of Ki-67 was only appeared upon invasive tumors such as malignant thyroid follicular tumors but not adenoma [26]. Combination of CyclinD1 and Ki-67 could identify ambiguous Hurthle cell adenoma from carcinoma [27].

Results of this test showed a positive correlation between P53 expression and risk of Hurthle cell tumor ($r = 0.561$, $p < 0.001$). Positive expression of P53 with extremely low risk was 29.6%, which was lower than groups of moderate risk ($X2 = 17.771$, $p < 0.001$) and high risk ($X2 = 11.165$, $p < 0.001$). There was 55.6% which highly expressing P53 with low risk and that might put up statistically significant to other groups if sufficient samples were collected. Similarly, the higher risk of tumor, the more positive of CyclinD1($r = 0.569$, $p < 0.001$). In each group, positive expression of CyclinD1 was 7.4%, 22.2%, 52.4% and 100%, respectively. Because of the limit cases, significance was merely shown off with extremely low risk and moderate/high risk, low risk and high risk.

Totally, there was only 0/27, 1/18, 2/21 and 2/7 cases which performed highly expressing of Ki-67 in each group. Ki-67 was reported [26] as a useful maker for distinguishing follicular adenoma from carcinoma in thyroid with specificity of 93% but low sensitivity. The reason was possibly that Ki-67 is not colored with G0 and prophase of G1 in cell proliferation thus reducing the positive detection of Ki-67.

Observing this study, we found out a tendency that the higher risk of Hurthle cell tumor, the more positive with P53, CyclinD1 and Ki-67. In other words, a tumor with high risk is more proliferative. A tumor which highly expressing Ki-67 was also positive stained with P53 and CyclinD1. At the same time, all samples that with up-regulated CyclinD1 were stained with P53. Abnormally gene regulation with tumors always occurs prior to morphological appearance. There was one case in low risk and two cases with moderate risk that highly expressed P53, CyclinD1 and Ki-67. The 3 tumors might be cut out in time before displaying malignant features. By following up, one of which displayed tumor regeneration with moderate risk. Another patient who suffered reoperation was also displayed positive of P53 and CyclinD1.

P53 gene mutation is an early event related to tumorigenesis. Tumors with abnormal P53 could continue to initiate the process of malignancy, or may not be invasive under regulation of the body. High expression of Ki-67 is the common endpoint on regulation of cell proliferation and considered as an independent predictor for Hurthle cell tumor [1]. We suggested that a Hurthle cell adenoma with high index of Ki-67 is aggressive and should enlarge the operation properly. We also recommended that Hurthle cell adenomas which negative expressing P53 could be treated as benign. Because of a long intermittence with tumor recurrence [5, 6], while a Hurthle cell adenoma with P53 positive expression, the patient should be rechecked for a long time especially with positive of CyclinD1. We also proposed that the homolateral gland of thyroid should be resected completely instead of rejecting the tumor only.

We put forward that a Hurthle cell tumor with higher risk is more proliferative. Detecting with P53, CyclinD1 and Ki-67 might provide reference for assessing the invasive potential with Hurthle cell tumor but not Galectin-3, COX-2 and Bcl-2.

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Authors Contribution Ding Li:design and specimen collecting; Jiang Yunhui:pathology diagnosis and specimen collecting; Yang Wan:operate a immunostaining.

Compliance with Ethical Standards

Competing Interests Each of the author has approved the final version of the manuscript and reports no conflicts of interest.

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