IGF2 EXPRESSION CAN BE A NEW INDEX IN THE DIAGNOSIS AND PROGNOSIS OF THYROID CARCINOMA

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ABSTRACT

Objective: *TTo investigate the expression of IGF2 in papillary thyroid carcinoma and its correlation with clinical pathology.*

Methods: *47 specimens of thyroid papillary carcinoma and 32 of normal thyroid tissues were collected from March 2012 to June 2013 in our hospital. The expression of IGF2 in thyroid papillary carcinoma and normal thyroid tissues was detected by immunohistochemistry and the relationship between its expression and clinical pathology was further analysed.*

Results: *The positive expression rate of IGF2 in the thyroid papillary carcinoma was 64.21% (30/47), compared with 25.81% (8/32) in the normal thyroid tissue was The expression of IGF2 in the patients with papillary carcinoma was related to the tumour diameter, lymph node metastasis and AJCC stage (P<0.05). It was not related to age (P>0.05). The 5-year survival rate of patients with high expression of IGF2 was 10.00% (3/30), which was significantly shorter than that of patients with low expression of IGF2 who had a 5-year survival rate of 47.06% (8/17).*

Conclusion: *IGF2 is highly expressed in papillary thyroid carcinoma, and its expression level is related to tumour diameter, lymph node metastasis and AJCC stage of thyroid papillary carcinoma. IGF2 expression can be seen as a new index in the diagnosis and prognosis of papillary thyroid carcinoma.*

Keywords: *IGF2, papillary thyroid carcinoma, clinical pathology, correlation.*

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Introduction

Thyroid papillary carcinoma is the most common clinicopathological type of thyroid cancer, accounting for approximately 85% of total thyroid cancer cases[1]. In recent years, there has been a gradual increase in the incidence of thyroid papillary cancer. According to statistics, the incidence of thyroid papillary cancer in the United States has increased threefold compared with the previous period, an increase primarily related to the discovery of tiny papillary cancer[2]. Thyroid papillary carcinoma is most commonly seen in children or young women, and its onset is influenced by factors including hormones, genetics and environment. A painless mass in the neck is the first symptom, and a few patients experience voice loss, dysphagia and a feeling of pressure[3].

Surgical resection is the preferred treatment for thyroid papillary cancer, as it is not sensitive to chemoradiotherapy[4]. Gene therapy is the key focus of current clinical research, which may provide a breakthrough in the treatment of thyroid cancer. It is especially important to further explore the pathogenesis of thyroid cancer and to develop an effective treatment plan. Insulin-like growth factors (IGFs) are a group of natural peptide hormones that promote growth and play an important role in human growth and development[5]. The IGF family includes IGF1, which plays a growth-promoting role in the form of autocrine or paracrine[6] and IGF2, which plays an important role in foetal growth and development, but on which there are relatively few clinical reports regarding its physiological effect and regulation mode[7].

Clinical studies have shown that surface receptors of IGF2 and IGF1 cells overexpressed in pancre-

atic cells promote the development of beta cells into tumours[8]. Existing studies have shown that IGF2 has the effect of driving sensitization of susceptible cell tumours[9]. The aim of this study is, therefore, to investigate the relationship between expression level and clinicopathological characteristics and prognosis of IGF2 in thyroid papillary carcinoma.

Materials and methods

General information

Pathological specimens were collected from 47 patients with thyroid papillary carcinoma who underwent surgical resection in our hospital from March 2012 to June 2013, including 13 males and 34 females. There were 27 patients <55 years old and 20 patients ≥55 years old. Lymph node metastasis was found in 22 cases and no lymph node metastasis in 25 cases. The tumour diameter was <2cm in 21 cases, ≥2cm in 26 cases. When categorised according to AJCC stage, 31 patients were in stage I~II and 16 in stage III~IV.

The inclusion criteria were as follows:

• All patients were diagnosed with thyroid papillary carcinoma by the relevant pathological examination and professional doctors;

• None had received any chemoradiotherapy before surgery;

• All patients signed informed consent;

• Approved by the ethics committee of the hospital.

The following patients were excluded:

• Those with incomplete clinicopathology;

• Those who had received anti-tumour treatment;

• Those suffering from autoimmune diseases;

• Those with abnormal heart, liver and kidney function. In addition, 32 specimens of normal thyroid tissue were selected, which were more than 2cm away from thyroid adenoma or nodules.

Main reagents and instruments

Reagents:

The conventional chemical reagent was purchased from Shanghai Sinopharm Biological Co. Ltd.; neutral gum from Sigma Company; PBS from Wellbio; two-step kit from Beijing Zhongshan Jinqiao Co. Ltd. Rabbit anti-human polyclonal antibody IGF2 was purchased from Bioss; 75% ethanol, 85% ethanol, 95% ethanol and 100% ethanol from Shanghai Kwanren Chemical Co. Ltd.; xylene from Jinan Junyuan Chemical Co. Ltd.

Instruments:

Shaking table was purchased from Instrument Manufacturing Co. Ltd.; microwave oven from the United States; slicer from Zhejiang Jinhua Yidi test equipment factory; ordinary refrigerator from Rongshida; microscope from Motic; slides from Haimen Yuantai Property Co. Ltd.

Methods

• Prepare 0.01mol/L PBS and citrate buffer.

• Immunohistochemical staining. Paraffin specimens were placed on a slicer for 4 microns continuous slicing and roasted in the oven for 1 hour to prevent peeling. The slices were immersed in xylene for 10 min, 5 min/time, repeated once, then placed in a buffer of citrate hydrochloric acid and heated until boiling. PBS solution was washed for 3 min, repeated 3 times. 3% H_2O_2 was added to block peroxidase activity. PBS solution was washed for 3 min, repeated 3 times. Primary antibody (IGF2) was added at 4oC overnight. PBS solution was washed for 3 min, repeated 3 times. The secondary antibody of rabbit IgG-horseradish peroxidase polymerase was added and placed at 37oC for 30 min. PBS solution was washed for 3 min, repeated 3 times. The prepared DAB chrominant was dropped into the section, incubated at room temperature for 5 min, and the chromination reaction was controlled at the same time, and washed with distilled water. Hematoxylin was redyed, washed with distilled water and PBS solution for 3 min, repeated 3 times. The slices were dehydrated in gradient ethanol. After removal, the slices were immersed in xylene for 10 min, 5 min/ time, and repeated once. After re-baking, the slices were sealed with neutral gum and observed under a microscope.

Immunohistochemical interpretation criteria

IGF2 is mainly expressed in the nucleus, cell membrane and cytoplasm, and the brown-yellow particles in the nucleus, cell membrane and cytoplasm are positively expressed.

According to the grading standard of staining intensity and proportion of positive cells, its expression can be divided into 4 grades:

• 0 points: no staining;

• 1 point: weak staining;

• 2 points: medium intensity staining;

• 3 points: strong staining. The H-score method was adopted for scoring, with ≤2 considered low expression and >2 high expression.

Statistical methods

SPSS23.0 was used for data analysis, and the chi-square test was used for differences between

groups. The survival rate was analysed by the Kaplan-Meier method, and the survival difference was tested by log-rank method. The Cox proportional risk regression model was used to evaluate the factors influencing the prognosis of patients, and P<0.05 was considered to be statistically significant.

Results

Expression of IGF2 in thyroid papillary carcinoma and normal thyroid tissues

The positive expression rate of IGF2 in thyroid papillary carcinoma was 64.21% (30/47), significantly higher than that in normal thyroid tissues (25.81% or 8/32) as shown in Figure 1.

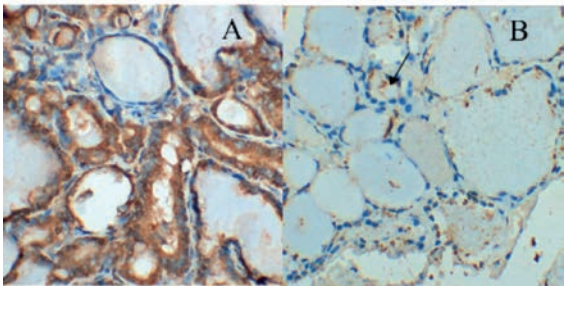

Figure 1: Expression of IGF2 in thyroid papillary carcinoma and normal thyroid tissues.
A: positive expression of IGF2 in thyroid papillary carcinoma; Figure B: positive expression of IGF2 in normal thyroid tissue.

Correlation between IGF2 expression level and clinicopathological characteristics of patients with thyroid papillary carcinoma

IGF2 expression level was correlated with tumour diameter, lymph node metastasis and AJCC stage in patients with thyroid papillary carcinoma (P<0.05). It was independent of gender and age (P>0.05). See Table 1.

Clinicopathological parameter	n	IGF2 expression		χ^2	*P*
		Positive expression (n=30)	Negative expression (n=17)		
Gender				0.948	0.39
Man	13	7 (23.33)	6 (35.29)		
Woman	34	23 (76.67)	11 (64.71)		
Age(year)				0.422	0.694
<55year	27	17 (56.67)	10 (58.82)		
≥55year	20	13 (43.33)	7 (41.18)		
Diameter of tumour (cm)				3.161	0.005
<2	21	10 (33.33)	11 (64.71)		
≥2	26	20 (66.67)	6 (35.29)		
Lymphatic metastasis				2.078	0.039
no	25	15 (50.00)	10 (58.82)		
yes	22	15 (50.00)	7 (41.18)		
AJCC				3.591	0.002
I~II	31	19 (63.33)	12 (70.59)		
III~IV	16	11 (36.67)	5 (29.41)		

Table 1: Correlation between IGF2 expression level and clinicopathological characteristics of patients with thyroid papillary carcinoma.

Correlation between IGF2 expression level and prognosis of patients with thyroid papillary carcinoma

The 5-year survival rate of patients with high IGF2 expression was 10.00% (3/30), significantly lower than that of patients with low IGF2 expression (47.06% (8/17), as shown in Figure 2.

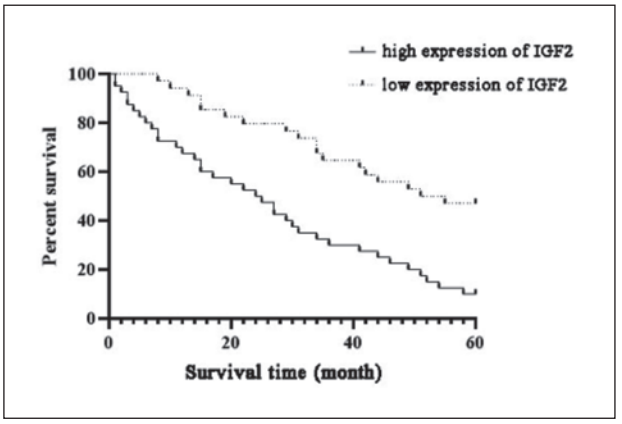

Figure 2: Correlation between IGF2 expression level and prognosis of patients with thyroid papillary carcinoma.

COX proportional risk regression model analysis for patients with thyroid papillary carcinoma

The COX proportional risk regression model analysis showed that lymph node metastasis, AJCC staging and IGF2 expression were independent risk factors affecting the prognosis of patients with thyroid papillary carcinoma, as shown in Table 2.

Clinicopathological parameter	Risk value	95% CI district	*P*
Age	0.523	0.322~0.855	0.598
Gender	0.815	0.522~1.275	0.355
Diameter of tumour	0.365	0.179~0.757	0.643
Lymphatic Metastasis	0.415	0.255~0.681	0.007
AJCC stage	0.911	0.584~1.422	<0.001
IGF2 expression	0.484	0.281~0.840	0.041

Table 2: Analysis of COX proportional risk regression model in patients with thyroid papillary carcinoma.

Discussion

Thyroid papilla carcinoma is a common malignant tumour of the thyroid gland, which can be identified by fine needle biopsy and cellular evaluation[10]. In recent years, diagnosis and treatment have improved greatly, but the specificity of these methods is not very high, and it is therefore particularly important to find a safe, effective and reliable biomarker. IGFs are multifunctional cell proliferation regulators that can promote cell differentiation,

proliferation and growth. They belong to the family of related secretory proteins[11] and act on the human body through autocrine, paracrine and remote secretion. They play an important role in promoting cartilage growth and stimulating mitosis of tissue cells. IGF2 is composed of 67 amino acids and is a weak acidic protein which is recognised as being instrumental in foetal metabolism, growth and division[12]. It is a more effective mitogen than IGF1. Clinical studies have shown that IGF2 only expresses paternal alleles in most tissues[13]. Under normal physiological conditions, the expression of IGF2 is accurate, but loss of imprinting can lead to abnormally high expression of IGF2, which can lead to disorders. Clinical studies have shown that IGF2 can induce vascular regeneration in mice through bone marrow monocytes, suggesting that it plays an important role in the growth of malignant tumours[14]. The expression of the IGF2 gene is regulated by many factors. Clinical studies have shown that insulin-like growth factor-2 mRNA binding protein 3 (IGF2/IMP3) can promote the growth of tumour cells by up-regulating IGF2 mRNA translation. Some scholars believe that IGF2 is closely related to the transformation of susceptible cells into tumour cells[15]. Clinical studies have shown that mice with IGF2 gene overexpression suffer from various types of tumour.

Firstly, we detected the expression of IGF2 in papilla thyroid carcinoma and normal thyroid tissue by immunohistochemical methods. The results showed a positive expression rate of IGF2 in papilla thyroid carcinoma of 64.21% (30/47), which was significantly higher than that in normal thyroid tissue of IGF2 (25.81%, 8/32). The correlation between the expression of IGF2 and the clinicopathological parameters of patients with papilla thyroid carcinoma was analysed according to the results of the immunohistochemistry. The results showed that the expression level of IGF2 was correlated with tumour diameter, lymph node metastasis and AJCC stage in patients with papilla thyroid carcinoma ($P<0.05$), but not with sex and age ($P>0.05$). In this study, the patients were followed up for up to 5 years, primarily by telephone follow-up and outpatient re-examination. The results showed that the 5-year survival rate of patients with high expression of IGF2 was 10.00% (3/30), which was significantly lower than that of patients with low expression of IGF2 (47.06%, 8 ≤17). The COX proportional risk regression model was established, and the results showed that lymph node metastasis, AJCC stage and IGF2 expression were independent risk factors in the prognosis of patients with papilla thyroid carcinoma.

In conclusion, IGF2 is highly expressed in papilla thyroid carcinoma, and its expression level is related to tumour diameter, lymph node metastasis and AJCC stage. Lymph node metastasis and AJCC stage are independent risk factors in the prognosis of patients with papilla thyroid carcinoma. IGF2 expression has a certain value in the diagnosis and prognosis of thyroid papilla carcinoma, and may become a new indicator of thyroid papilla carcinoma.

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