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Expression characteristics of proteins of IGF-1R, p-Akt, and survivin in papillary thyroid carcinoma patients with type 2 diabetes mellitus

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Abstract

Type 2 diabetes mellitus (T2DM) is related to increased risk of papillary thyroid carcinoma (PTC). Insulin-like growth factor-1 receptor (IGF-1R) is increased in patients with T2DM. The increased IGF-1R may be responsible for the development of PTC. In this study, we investigated the expression of phosphorylation of Akt (p-Akt)/survivin pathway activated by IGF-1R in PTC subjects with and without diabetes.

Clinicopathological data of 20 PTC patients with T2DM were retrospectively analyzed and compared with those of 21 PTC subjects without diabetes. Meanwhile, IGF-1R, p-Akt, and survivin expressions of PTC tissues were detected by immunohistochemical staining.

The immunohistochemical results found that the expression level of IGF-1R was significantly higher in diabetic PTC patients than that in nondiabetic PTC patients ($P < 0.05$). However, no significant differences of p-Akt and survivin expression were found between PTC patients with T2DM and PTC patients without T2DM. In addition, among 20 PTC patients with T2DM, subgroup analysis showed that the ratio of tumor size >10 mm was significantly higher in IGF-1R moderate to strong expression group than that in IGF-1R negative to weak expression group ($P < 0.05$).

IGF-1R expression level was higher in PTC patients with T2DM, and the increased IGF-1R expression was associated with lager tumor size. IGF-1R may play an important role in carcinogenesis and tumor growth in PTC patients with T2DM.

Abbreviations: BMI = body mass index, IGF-1R = insulin-like growth factor-1 receptor, MAPK = mitogen-activated protein kinase, p-Akt = phosphorylation of Akt, PI3K = phosphatidylinositol 3-kinase, PTC = papillary thyroid carcinoma, T2DM = type 2 diabetes mellitus.

Keywords: IGF-1R, p-Akt, papillary thyroid carcinoma, survivin, type 2 diabetes mellitus

1. Introduction

During the past several decades, an increasing incidence of papillary thyroid carcinoma (PTC) has been observed in several countries. A study conducted in 5 continents reported that the average increase of incidence rate of thyroid cancer is 48.0% among males and 66.7% among females over the 30-year period (1973–2002).[1] In China, the incidence rate of thyroid cancer is rising by about 14.5% per year.[2] The general applications of thyroid ultrasonography and some other factors may contribute to the increased incidence and prevalence of thyroid cancer.[3]

Meanwhile, a parallel secular rise in type 2 diabetes mellitus (T2DM) prevalence and morbidity has been reported. The World Health Organization predicted that in 2030 the total diabetic population would rise to 300 million.[4] Furthermore, higher risks and mortalities of a variety of cancers have been reported in diabetes.[4,5] Similarly, a higher risk of thyroid carcinoma is found in T2DM females than in the general females.[6] A 10-year prospective study in America reported that the risk of thyroid carcinoma increases 25% among diabetics.[7] It is well known that insulin resistance and hyperinsulinemia is the key feature of T2DM, while insulin resistance and hyperinsulinemia is associated with cancers, for example, breast and colon carcinoma.[5] However, the exact biological mechanisms underlying the carcinogenic effects of T2DM in PTC are not fully investigated and reported. We speculated that there may be distinctive pathogenesis of thyroid carcinoma in patients with T2DM, which is different from that in those without T2DM.

The insulin-like growth factor-1 receptor (IGF-1R) plays important roles in insulin resistance and hyperinsulinemia in diabetes, and its expression is significantly higher in lung cancer with T2DM.[8] IGF-1R activation by tyrosine phosphorylation of β-subunit results in activation of phosphatidylinositol 3-kinase (PI3K)/Akt and RAS/mitogen-activated protein kinase (MAPK) pathways that in turn regulate cell survival and proliferation.[9,10] The phosphorylation of Akt (p-Akt) regulates expression of survivin via promoting hypoxia-inducible factor-1a expression. Hypoxia-inducible factor-1a binds to survivin promoter, and then promotes survivin transcription and translation; survivin is

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1 member of inhibitor of apoptosis protein family to inhibit apoptosis in cancers.[11,12] Previous studies reported that p-Akt and survivin were significantly expressed in thyroid carcinoma,[13–15] whereas it remains unknown whether IGF-1R/p-Akt/survivin pathway is involved in the pathogenesis of PTC in diabetes or not. In this study, we compared the immunohistochemical expression and intensity of IGF-1R, p-Akt, and survivin in PTC patients with or without T2DM to investigate the mechanistic link between diabetes and PTC.

2. Materials and methods

2.1. Patients

Data on patients with PTC who were admitted to West China Hospital of Sichuan University from January 2009 to July 2014 were retrospectively searched. Inclusion criteria were as follows: PTC confirmed by histology or cytology, patients who had undergone thyroidectomy, patients older than 18 years, patients who have not undergone radiotherapy or chemotherapy before surgery, and patients with pre-existing T2DM before the diagnosis of PTC. Excluding criteria were as follows: coexistence of another type of thyroid malignancies, other recurrent or concurrent malignancies, a history of other neck surgery or radiation, a history of ^{131}I treatment, type 1 diabetes mellitus, and diagnosis of diabetes mellitus or hyperglycemia after hospitalization.

To compare PTC patients with T2DM, we searched those PTC patients without the diagnosis of diabetes from the database whose age, gender, and the stage of tumor were not statistically different by χ^2 test and rank-sum test compared with those of the T2DM group. All PTC patients without diabetes should be verified for the same inclusion and exclusion criteria mentioned earlier except for the diagnosis of T2DM, and the nondiabetic patients should have a morning fasting blood glucose <5.6 mmol/L.

2.2. Tissue samples

Tumor tissues were acquired from formalin-fixed pathological samples taken from the resected PTC specimens. Clinicopathological characteristics of patients with and without T2DM were collected. The study was approved by the Biomedical Ethics Committee of West China Hospital, Sichuan University, China.

2.3. Immunohistochemical staining

The tissue samples of PTC patients were cut into sections of 4 μm, which were mounted on silanized slides. All primary antibodies were purchased from R&D America, and belonged to polyclonal: IGF-1R (AF-305-NA) at 1:100, p-Akt (AF887) at 1:100, and survivin (AF886) at 1:100. Two-step immunohistochemistry detection reagents were PV9003 from ZSJQ-BIO (Beijing, China) and K5007 from Dako (Glostrup, Denmark).

2.4. Evaluation of immunohistochemical staining results

Expressions of all antigens were examined by 2 investigators who were blinded to clinical data of the patients. Each sample was examined in a high-power field at ×400 magnification. The evaluation of staining was referred to a previous report with some modifications, as follows: immunostaining was classified based on staining intensity and percentage of positive tumor cells. Staining intensity was determined as 0 (absent), 1 (weak), 2 (moderate), and 3 (strong).[8,16] To compare the positive degree of antigen expression between the 2 groups, expression levels of the antigens were semiquantified using an immunohistochemistry score (range, 0–300) calculated by multiplying staining intensity with the percentage of positive tumor cells. The immunoreactivity was classified as follows: 0, score ≤60; 1+, 60 < score ≤ 140; 2+, 140 < score ≤ 220; and 3+, 220 < score ≤ 300. Patients with an immunohistochemistry score of ≤140 were considered as having negative to weak immunoreactivity and those with a score of >140 as having moderate to strong immunoreactivity, which will be used for the subsequent multivariate analysis.

Table 1

Clinicopathological data for PTC patients with and without T2DM.

Variable	PTC with T2DM (n = 20)	PTC without T2DM (n = 21)
Gender		
Male	9	10
Female	11	11
Age, y		
≤45	1	1
>45	19	20
BMI, kg/m^2		
≤24	8	14
>24	12	7
Hypertension		
Y	11	3
N	9	18
Tumor stage		
I	4	3
II	0	0
III	9	11
IV	7	7
Lymph node metastasis		
Y	7	10
N	13	11
Tumor size, mm		
≤10	7	10
>10	13	11
Extrathyroid invasion		
Y	17	16
N	3	5

BMI = body mass index, N = no, PTC = papillary thyroid carcinoma, T2DM = type 2 diabetes mellitus, Y = yes.

2.5. Statistical analysis

Statistical analysis was performed using SPSS 16.0 (SPSS Inc, Chicago, IL). Chi-square and Fisher tests were used for comparison between clinicopathological characteristics of the 2 groups. Rank-sum test was used to compare differences of antigen expression between the 2 groups. Spearman rank correlation and κ consistency test were used to compare staining intensity between antigens. A P value of <0.05 was considered statistically significant.

3. Results

3.1. Clinicopathological characteristics

This study included 20 PTC subjects with T2DM and 21 PTC individuals without T2DM. As shown in Table 1, the ratio of hypertension was higher in PTC patients with T2DM than that in PTC subjects without T2DM. There were no significant differences in age, gender, body mass index (BMI), tumor size, pTNM

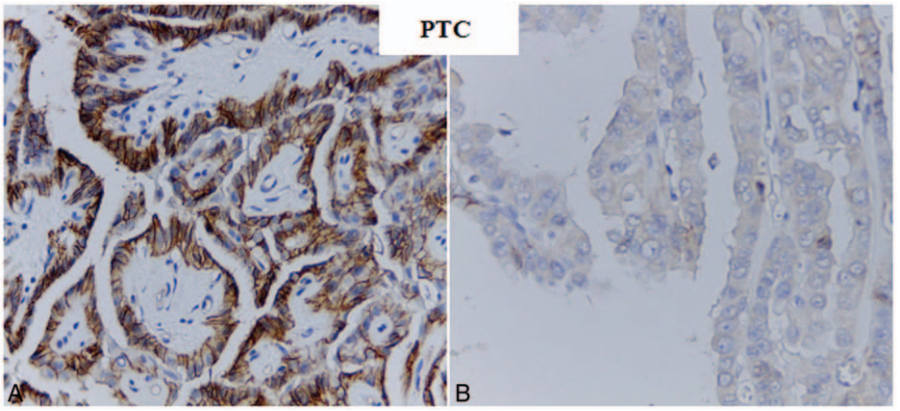

Figure 1. The representative figures of expression of IGF-1R in PTC (×400). IGF-1R = insulin-like growth factor-1 receptor, PTC = papillary thyroid carcinoma.

status, lymph node metastasis, and extrathyroid invasion in PTC subjects with and without T2DM (Table 1).

3.2. Expression differences of IGF-1R, p-Akt, and survivin in PTC patients with or without T2DM

IGF-1R staining in the PTC patients with T2DM was observed to be obviously stronger than that in the PTC patients without T2DM (mean rank 24.73 vs 17.45, $P=0.037$). No statistically significant differences were found in the expression of p-Akt and survivin between PTC patients with or without T2DM, as shown in Table 2. The representative pictures of immunohistochemistry of these molecules are shown in Figs. 1 to 3.

3.3. Expression correlation and consistency among IGF-1R/Akt/survivin proteins in PTC patients with T2DM

In 20 PTC patients with T2DM, p-Akt expression was consistent with survivin expression by κ analysis ($k=1.000$, $P<0.001$). IGF-1R expression was not consistent with p-Akt or survivin expression (IGF-1R and p-Akt: $k=0.318$, $P=0.144$; IGF-1R and survivin: $k=0.318$, $P=0.144$). Spearman rank analysis showed that the expression intensity of p-Akt was positively correlated with survivin (correlation coefficient 0.820, $P<0.001$). However, IGF-1R expression was not correlated with p-Akt or survivin (all $P>0.05$).

3.4. The correlation of clinicopathological factors with the expression of IGF-1R in PTC patients with T2DM

In order to investigate whether the immunoreactivity of IGF-1R was associated with TNM stages or tumor size, the immunoreactivity of IGF-1R was classified into IGF-1R negative to weak immunoreactivity group and IGF-1R moderate to strong immunoreactivity group according to immunohistochemistry score mentioned earlier. As shown in Table 3, the ratio of tumor size >10 mm was significantly higher in IGF-1R moderate to strong immunoreactivity group than in IGF-1R negative to weak immunoreactivity group (Fisher test, $P=0.007$).

4. Discussion

In this study we compared IGF-1R expression in PTC patients with and without diabetes to investigate the potential mechanistic link between diabetes and PTC, and the data showed that in PTC tissues IGF-1R was significantly highly expressed in diabetics than in nondiabetics. A previous study reported that IGF-1R expression is significantly higher in non-small cell lung cancer patients with preexisting T2DM.[8] Significantly higher level of IGF-1R messenger ribonucleic acid is observed in colorectal cancer tissue in patients with T2DM when compared with that in subjects without T2DM.[17] The higher expression of IGF-1R involved in diabetes affecting carcinogenesis may be associated

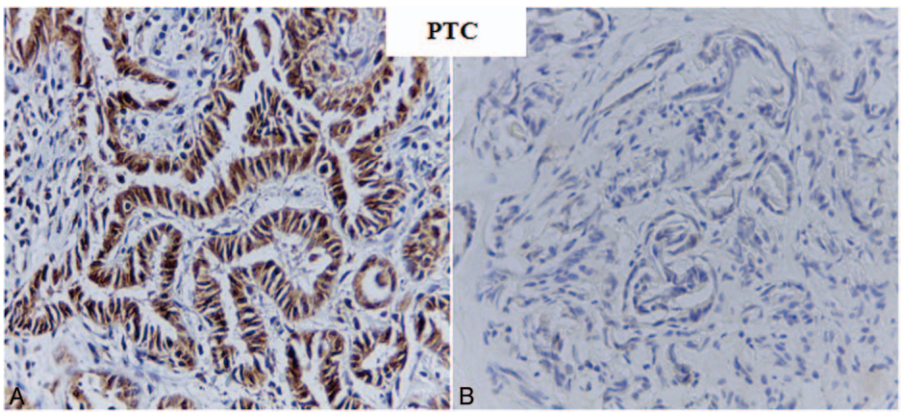

Figure 2. The representative figures of expression of p-Akt in PTC (×400). p-Akt = phosphorylation of Akt, PTC = papillary thyroid carcinoma.

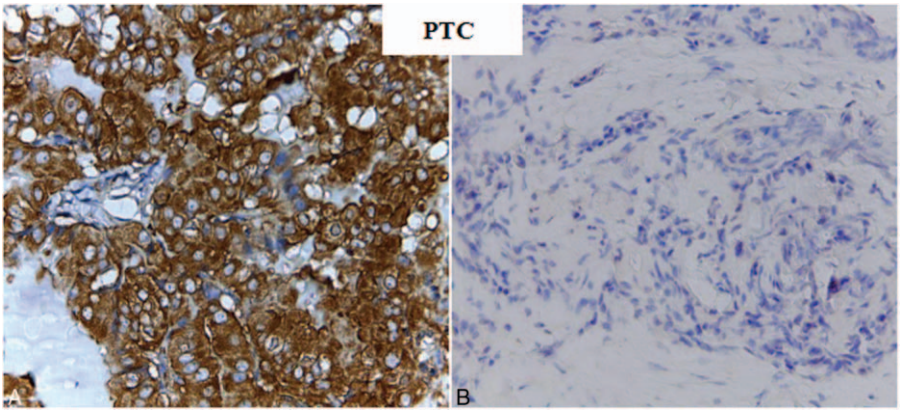

Figure 3. The representative figures of expression of survivin in PTC (×400). PTC = papillary thyroid carcinoma.

with hyperinsulinemia and hyperglycemia accompanied with diabetes. In T2DM patients, hyperinsulinemia reduces liver IGF-1-binding-protein synthesis, and increases serum level of free IGF-1 that integrates with IGF-1R and then activates downstream signaling pathway to facilitate cell proliferation and stimulate cancer cell growth.[18–20] In addition, hyperglycemia characterized with diabetes directly or indirectly promotes IGF-1R phosphorylation (activation) by stimulating advanced glycation end product production to facilitate tumor cell proliferation.[21,22] Our results indicated that IGF-1R may mediate the mechanism of PTC carcinogenesis in diabetes.

To further investigate the intracellular signaling pathway activated by IGF-1R in PTC patients with T2DM, we analyzed the expressions of p-Akt and survivin, the IGF-1R downstream molecules in PTC patients with and without diabetes. The immunohistochemical results showed that p-Akt and survivin expressions were not significantly different between PTC patients with and without diabetes. Although survivin expression was highly consistent and positively correlated with p-Akt expression, no significant consistency or correlation between IGF-1R and p-Akt or survivin expression was found in patients with diabetes. The results suggested that the p-Akt/survivin pathway may not be involved in diabetes affecting PTC. IGF-1R activation by tyrosine phosphorylation of β-subunit results in activation of PI3K/Akt and RAS/MAPK pathways that in turn regulate cell survival and proliferation, respectively.[9,10] Moreover, a previous study found that the activation of MAPK signaling by insulin/IGF-1 is responsible for the proliferation of colon cancer cells with T2DM.[23] Diabetes mellitus stimulates pancreatic cancer growth via the MAPK pathway.[24] High glucose increases glucose uptake and glycolytic activity, and stimulates cell proliferation through modulating the MAPK pathway.[25] Apart from PI3K/Akt pathway, MAPK pathway should be suspected to activate and greatly implicate in PTC development.[26] Even if there has been no research to investigate the MAPK pathway in PTC patients with T2DM, it is notable that MAPK pathway may be involved in diabetes affecting PTC. In PTC patients with diabetes, the percentage of tumor >10 mm was significantly higher in IGF-1R moderate to strong immunoreactivity group compared with that in IGF-1R negative to weak immunoreactivity group. The positive relationship between PTC size and IGF-1R expression in diabetes suggested that IGF-1R may promote tumor growth via activating cell proliferation pathway. However, this suggestion and the specific mechanisms and pathways activated by IGF-1R in PTC patients with T2DM need to be further demonstrated and investigated.

In addition, this study incidentally found that the percentage of BMI >24 kg/m^2 was higher in PTC patients with diabetes than in PTC patients without diabetes. Similarly, the ratio of hypertension in diabetics was significantly higher than that in nondiabetics (55.00% vs 14.29%) in this study. The relationship between obesity, hypertension, insulin resistance, and hyperinsulinemia is consanguineous.[27–33] That the diabetics had higher ratio of BMI >24 kg/m^2 or hypertension than nondiabetics was a limitation of this study; however, the most significant baseline characteristics, for example, number of cases, TNM stage, and tumor size, were consistent in patients with and without diabetes, and hence the results were persuasive and convinced.

5. Conclusions

In conclusion, this study found that in PTC patients with T2DM, IGF-1R was overexpressed when compared with that in subjects without T2DM, and IGF-1R immunoreactivity was associated

Table 2

The expression differences of IGF-1R/p-Akt/survivin in PTC with and without T2DM.

	IGF-1R		p-Akt		Survivin	
	PTC with T2DM	PTC without T2DM	PTC with T2DM	PTC without T2DM	PTC with T2DM	PTC without T2DM
0	3	6	2	2	2	4
1+	1	6	0	0	1	0
2+	3	2	4	3	3	6
3+	13	7	14	16	14	11
Mean rank	24.73	17.45	20.40	21.57	19.29	22.80
P value	0.037		0.687		0.281	

IGF-1R = insulin-like growth factor 1 receptor, p-Akt = phosphorylation of Akt, PTC = papillary thyroid carcinoma, T2DM = type 2 diabetes mellitus.

Table 3

The IGF-1R expression and clinicopathological differences in PTC with T2DM.

	Negative to weak expression (n = 4)	Moderate to strong expression (n = 16)	*P* value
Age, y			
≤45	0	1	>0.05
>45	4	15	
Gender			
Male	2	7	>0.05
Female	2	9	
BMI, kg/m^2			
≤24	0	8	>0.05
>24	4	6	
Hypertension			
Y	2	9	>0.05
N	2	7	
Lymph node metastasis			
Y	1	6	>0.05
N	3	10	
Tumor stage			
I–II	0	4	>0.05
III–IV	4	12	
Tumor size, mm			
≤10	4	3	<0.05
>10	0	13	
Extrathyroid invasion			
Y	4	13	>0.05
N	0	3	

BMI = body mass index, IGF-1R = insulin-like growth factor 1 receptor, N = no, PTC = papillary thyroid carcinoma, T2DM = type 2 diabetes mellitus, Y = yes.

with tumor size. It is suggested that IGF-1R may be involved in diabetes affecting tumorigenesis and tumor growth of PTC. However, further research is needed.

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