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Original Article

Over-expression of GOLPH3 is related to poor prognosis in thyroid papillary carcinoma patients

Zhi-Dong Lin*, Dan Tang*, Geng-Long Zhu, Jin-Lin Zou, Qing-An Zeng

*Department of General Surgery, The Fifth Affiliated Hospital of Sun Yat-Sen University, Zhuhai, Guangdong, P. R. China. *Equal contributors.*

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Abstract: Background: Thyroid papillary carcinoma (TPC) is a common malignant endocrine tumor. Although patients with TPC show favorable long-term survival, frequent lymph node relapses occur in the early disease stage. Overexpression of golgi phosphoprotein 3 (GOLPH3) has been associated with poor prognosis in patients with solid malignant tumors. We evaluated the relationship between tumoral GOLPH3 expression and survival in patients with early stage TPC. Methods: GOLPH3 mRNA and protein expression were determined using real-time polymerase chain reaction and western blot analysis in patient-derived TPC and nodular goiter tissues. Tissue samples were obtained from 189 patients with early stage TPC who underwent surgical resection between 2004 and 2008. Immunohistochemical GOLPH3 expression pattern was also evaluated. Statistical correlations between GOLPH3 expression, clinicopathological characteristics, and prognosis were evaluated. Result: The mean GOLPH3 mRNA and protein levels were significantly greater in TPC tissues compared with nodular goiter controls. GOLPH3 protein expression in TPC tissues was significantly associated with postsurgical pathological T phase, N phase, and relapse rate. High GOLPH3 expression in patients with TPC was associated with tumor capsule invasion, lymph node metastasis, and surgery. Conclusion: As high GOLPH3 expression in patients with TPC was associated with tumor capsule invasion, lymph node metastasis, and surgery, such features may be useful for selecting patients with TPC who might benefit from treatments that are more aggressive.

Keywords: Thyroid papillary carcinoma, GOLPH3, relapse, prognosis

Introduction

Thyroid papillary carcinoma (TPC) comprises ~1% of all tumors and is the most frequent malignant endocrine tumor. Global TPC incidence has increased by ~5%, accounting for the highest cancer incidence increase in women and the second highest in men [1, 2]. The papillary thyroid cancer (PTC) subtype represents 80-90% of all TPCs. PTC is a low-grade malignant tumor characterized clinically by a slowly growing thyroid mass, potential multiple lesions, and occult lymph node metastasis. Conventional treatments for PTC include surgery, isotope treatment, thyrotropin suppression therapy, and auxiliary radiotherapy, with surgery being the standard treatment option. PTC has good prognosis with 5-year and 10-year OS rates of 95% [3] and 90% [4], respectively. Despite this, 10% of patients experience relapse, metastasis, and death [5]. Neck lymph node metastasis, especially in the VI cervical region, is the most frequent type of relapse [6, 7]. Preventative neck lymph node dissection for cT1-2N0 PTC delays relapse due to tumor metastasis, but is associated with complications such as hypoparathyroidism, hoarseness, choking/aspiration, lymphatic fistula, and esophageal fistula.

Many studies have focused on screening for high-risk PTC to better select patients for targeted treatment and lower the need for unnecessary surgeries that might affect patient quality of life. In addition to TNM staging, multiple institutes have attempted to grade PTC based on biological research. The American Thyroid Association has suggested alternative surgeries and postoperative treatments based on biological studies and the presence of high-risk clinical factors [8].

The *GOLPH3* gene, also known as GPP34/GMx33/MIDAS, is located on chromosome

Table 1. Clinicopathological characteristics and GOLPH3 expression in patients with papillary thyroid cancer

Characteristics	Number of cases (%)
Sex	
Male	28 (14.8)
Female	161 (85.2)
Pathologic T stage	
T1	108 (57.1)
T2	52 (27.5)
T3	29 (15.3)
Envelope invasion	
No	160 (84.6)
Yes	29 (15.4)
Pathologic N stage	
N0	117 (61.9)
N1a	58 (30.7)
N1b	14 (7.4)
Recurrence	
No	153 (81.0)
Yes	36 (19.0)
Vital status (at follow-up)	
Alive	189 (100)
Dead	0 (0.00)
GOLPH3 expression	
Low	96 (50.8)
High	93 (49.2)

5pl3. Golgi phosphoprotein 3 (GOLPH3) is a phosphorylated golgi membrane protein located in the transgolgi network that is involved in the protein-sorting pathway. GOLPH3 has been suggested to possess strong oncogenic potential [9, 10], although its role in oncogenesis has not been elucidated fully. The transgolgi network mediates protein sorting, packaging, modification, and transportation [11] indicating that GOLPH3 may be involved in oncogenesis via these functions.

Abnormal GOLPH3 expression has been closely associated with poor prognosis in patients with breast [12], esophageal [13], gastric [14], tongue [15], and prostate [16] cancers, and in those with gliocytoma [17]. Previous GOLPH3 functional studies have included investigations involving the transforming growth factor, Hedgehog, mitogen-activating protein kinase, and p53 tumor suppressor signaling pathways, which have been implicated in diverse cellular and potential oncogenic processes such as cell motility, cell adhesion, cell-extracellular matrix interaction, and cell cycle regulation [18]. Furthermore, a preclinical study demonstrated that GOLPH3 mRNA knockdown significantly reduced the growth and metastases of esophageal squamous cancer in vivo [19].

PTC development is multifactorial and multigenic. Current research is focused on the role of GOLPH3 in promoting oncogenesis and thyroid hyperplasia, differentiation, and metastasis. The aim of the present study was to evaluate GOLPH3 expression in patients with PTC, and its association with clinicopathological characters and prognosis, for improved patient selection and treatment planning.

Materials and methods

Patients

Patients or family members provided informed consent for the use of patient data and tissue samples. The present study was approved by the Ethics Committee of the Fifth Affiliated Hospital, Sun Yat-sen University. Initially, 197 patients with cN0 PTC were diagnosed and underwent surgery at our hospital with any presurgical chemotherapy or radiotherapy. Eight matched pairs of PTC and nodular goiter tissues were obtained for reverse-transcription polymerase chain reaction (RT-PCR) and western blot analysis. Paraffin-embedded PTC sections were obtained from 189 patients (161 women: 28 men) treated in our hospital between 2004 and 2008. All patients were confirmed as PTC by clinical diagnosis and pathological assays.

In all patients, follow-up exceeded 5 postsurgical years, with a median follow-up of 102 months (range, 65-132 months). All T1-2 patients underwent unilateral thyroid lobe and isthmus resection. Intraoperative frozen-section analysis was performed to determine patients indicated for lymph node clearance in the VI and lateral neck regions. Patient clinicopathological characteristics and tissue GOLPH3 immunohistochemical details are summarized in **Table 1.**

Western blot analysis

Protein was extracted from 8 matched pairs of PTC and nodular goiter (control) tissues. Protein

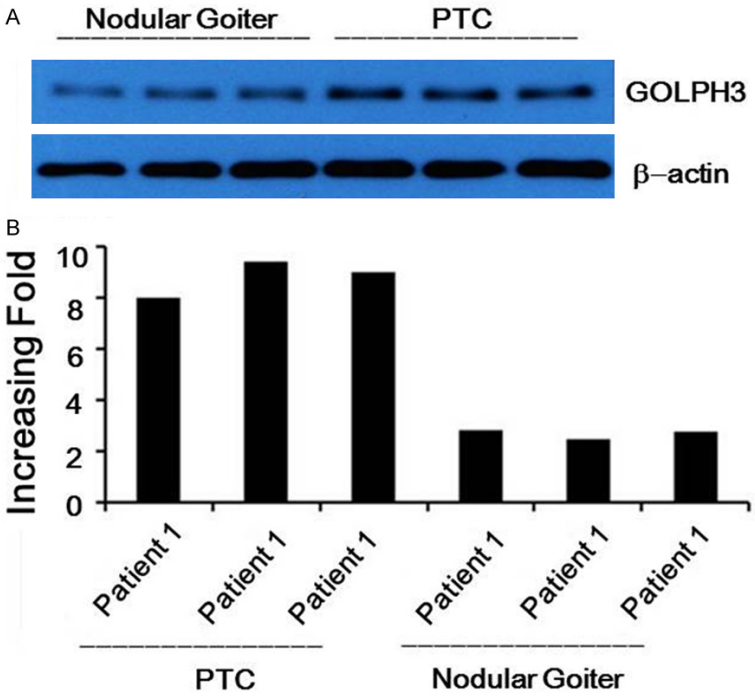

Figure 1. GOLPH3 protein and mRNA in papillary thyroid cancer and nodular goiter tissues (A, B).

concentration was determined using a BCA assay (manufacturer details). Protein [amount] was loaded on [%] polyacrylamide gels for SDS-PAGE and gels were run at [volts] on [apparatus] in [Buffer]. Blots were transferred to PVDF membranes [manufacturer] using the Bio-Rad mini transfer system at 100 mA for 3 hours at 4°C in [buffer] [supplier]. Membranes were blocked with 5% non-fat milk TBST solution and incubated for 1-1.5 hours at room temperature. Membranes were subsequently incubated in GOLPH3 antibody at 4°C overnight (rabbit anti-human GOLPH3, Abcam, 1:1500 dilution) or at room temperature for 1 hour in β-actin mouse monoclonal antibody (Sigma, 1:1000 dilution). After washing in [buffer], membranes were incubated with a secondary anti-mouse or anti-rabbit horseradish peroxidase antibody [supplier details] for [time] at [temperature]. Protein signals were detected by membrane incubation in chemiluminescent reagent for 1 minute, followed by radiographic film exposure and scanning.

Reverse transcriptase-polymerase chain reaction

RNA was extracted from 8 matched PTC and nodular goiter tissues using TRIzol reagent, according to manufacturer's instructions (Invitrogen), and treated with RQ1 RNase-free DNase (Promega) before cDNA synthesis using the iScript™ cDNA Synthesis Kit (Bio-Rad Laboratories). A semi-quantitative PCR assay was performed for GOLPH3 using β-actin as an internal control. The GOLPH3 and β-actin primers were designed using Primer Express Software version 2.0 (Applied Biosystems). PCR reaction steps were as follows: 95°C preheating 2 minutes, 40 cycles of 95°C denaturing for 15 seconds, 60°C annealing for 45 seconds, and 72°C extension for 1 minute. All experiments were performed in triplicate.

Immunohistochemistry

Immunohistochemical assays were performed using [kit details] according to manufacturer's instructions [manufacturer]. Briefly, paraffin-embedded sections were rehydrated and microwaved to restore the antigen exposure before incubation in 3% H_2O_2 at room temperature to ablate endogenous catalase activity. Sections were then washed with PBS and incubated in GOLPH3 antibody (1:100 dilution) at 4°C overnight, followed by washing in PBS, and incubation in enhancer solution for 20 minutes at room temperature. Sections were then incubated in conjugated secondary antibody for 30 minutes at room temperature and subsequently washed with PBS. This routine procedure was performed with DAB and hematoxylin staining, 0.1% HCl differentiation, PBS washing, and dehydration before neutral resin mounting was performed. Positive controls were provided with the kit and negative controls were obtained by omission of the primary antibody.

GOLPH3 positive staining was confined to the cytoplasmic area and scored as follows: 0, no staining; 1, light yellow staining; 2, brownish-yellow staining; and 3, dark brown staining. In addition, positive cell counts were scored as follows: 1, 1-10% positive; 2, 11-50% positive; 3, 51-75% positive; and 4, >75% staining. A total score was obtained by multiplying the

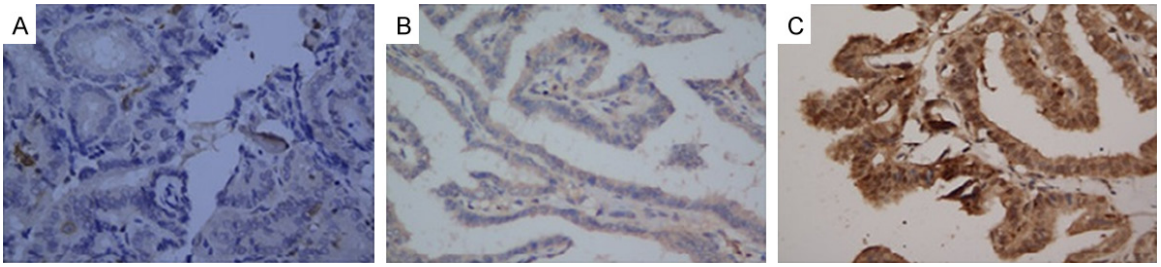

Figure 2. Immunohistochemical staining for GOLPH3 in patient-derived papillary thyroid cancer tissues (A-C). Magnification ×400.

Table 2. Correlation between GOLPH3 expression and clinicopathological characteristics of patients with cN0 thyroid papillary carcinoma

Characteristics	n	GOLPH3 expression		χ^2 test P (Fisher's exact test P)
		Low or none, n (%)	High, n (%)	
Sex				0.141
Male	28	12 (42.9)	16 (57.1)	
Female	151	84 (55.6)	77 (45.4)	
Pathologic T stage				0.026
T1	108	60 (55.6)	48 (44.4)	
T2	52	30 (57.7)	22 (42.3)	
T3	29	6 (20.7)	23 (79.3)	
Envelope invaded				0.001
No	160	87 (54.3)	73 (45.7)	
Yes	29	9 (31.0)	20 (69.0)	
Pathologic N stage				0.006
N0	117	74 (63.2)	43 (36.8)	
N1a	58	21 (36.2)	37 (63.8)	
N1b	14	1 (7.1)	13 (92.9)	
Recurrence				0.001
No	153	87 (61.0)	66 (39.0)	
Yes	36	9 (26.8)	27 (73.2)	

results of these two scoring systems. A total score >2 was considered positive expression; <4 was low expression and >6 was high expression.

Statistical analyses

All statistical analyses were conducted using SPSS version 13.0. Pearson's Chi squared and Fisher's exact tests were used for correlation analysis between GOLPH3 expression and clinicopathological phenotypes. Kaplan-Meier survival curves were produced for survival analysis. The Cox regression model was used for univariate and multivariate factor analysis. A *p* value of <0.05 was considered statistically significant.

Results

According to western blot analysis, GOLPH3 protein expression was greater in the PTC samples compared with the nodular goiter samples (**Figure 1A**). In addition, GOLPH3 mRNA expression was 2.4-4.1-fold greater in the PTC tissues compared with the nodular goiter samples (**Figure 1B**).

A comparison of GOLPH3 immunohistochemical staining with clinicopathological features of PTC is shown in **Figure 2** and **Table 2**. Of 189 cases, 182 (96.3%) were positive for GOLPH3; expression was high in 93 cases (49.2%) and low in 96 cases (50.8%).

GOLPH3 expression level was significantly associated with postsurgical conditions including T phase (P = 0.026), N phase (P = 0.001), capsule integrity (P = 0.006) and relapse (P<0.001). Therefore, compared with low GOLPH3 expression, high GOLPH3 expression predisposed the PTC patient to lymph node metastasis, capsule invasion, and relapse.

Survival analysis indicated that GOLPH3 expression was negatively correlated with event-free survival. In PTC patients 5-year event-free survival was 90.6% and 70.9% (P<0.001) for PTC patients with low and high GOLPH3 expression, respectively (**Figure 3**). COX risk model analysis revealed that GOLPH3 expression (relative risk, 2.533; confidence interval [CI], 1.379-4.652; P = 0.003), capsule invasion (relative risk, 3.027; CI, 2.451-5.738; P

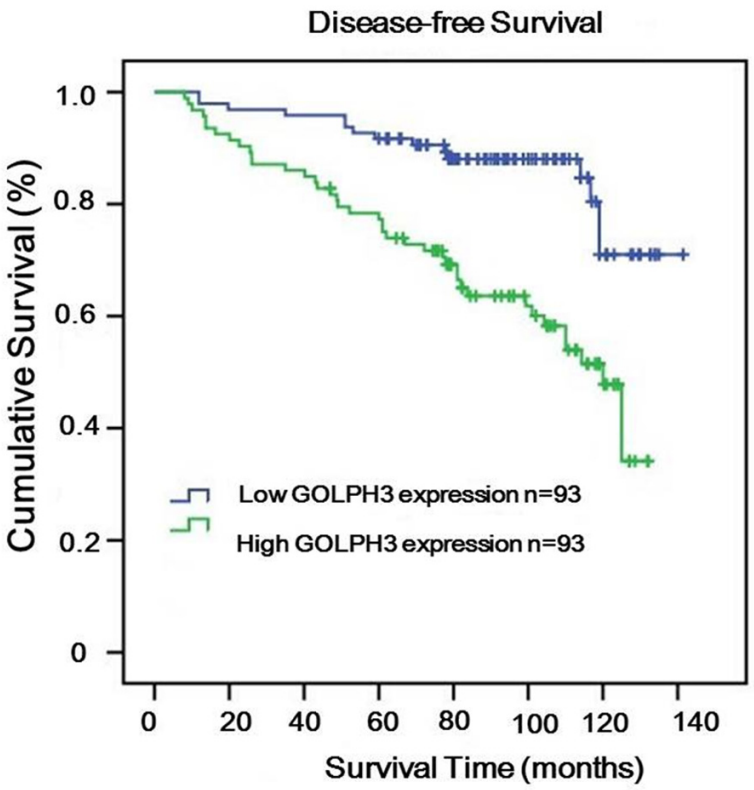

Figure 3. Kaplan-Meier analysis of GOLPH3 expression and postsurgical relapse in patients with papillary thyroid cancer.

= 0.016), and lymph node metastasis (relative risk, 2.568; CI, 1.465-4.499; P = 0.001) were associated with poor prognosis.

Discussion

The incidence of PTC has increased in recent years. Although the general prognosis for PTC is good, invasive and distal metastases of PTC have been associated with fatalities [20]. The clinical goal for patients with PTC is an improvement in quality of life without any adverse effects on long-term survival. With this in mind, the first choice treatment should be based on screening of patients with a high-risk of relapse or metastases. Previous studies have indicated that distal metastasis, patient age, and local infiltration are common prognosticators in patients with PTC [21-23]. Tumor markers for the clinical diagnosis and prognosis of cancers have been identified, but few are applicable for patients with PTC. The present study showed that GOLPH3 expression was clearly greater in PTC tissues compared with nodular goiter tissues. In addition, the 5-year event-free survival rate in patients with high GOLPH3 expression was significantly shorter compared with that in patients with low GOLPH3 expression. Furthermore, GOLPH3 expression correlated with T phase, N phase, local infiltration, lymph node metastasis, and relapse. These findings suggest that GOLPH3 expression could represent a suitable biomarker to screen for high-risk PTC patients, especially considering that those with high GOLPH3 expression were significantly more susceptible to relapse compared to those with low expression. In addition, GOLPH3 expression correlated with capsule invasion and lymph node metastasis, which were poor prognosticators of PTC.

Opinions on the contribution of sex and age to PTC relapse differ. It is reasonable to assume that age would be an important factor in considering PTC patient screening. The Chinese Nodular Goiter and Differentiated Thyroid Cancer Guidelines [24] suggest that 45 years-old is a critical age when screening PTC patients. To exclude confounding factors associated with age, the present study enrolled adult patients <45 years-old. Cunningham et al [25] suggested that sex was associated with relapse in patients with PTC.

In the present study, univariate analysis indicated that the relapse rate was higher in men compared to women, although this association was not noted on the multivariate analysis. This might be because of the imbalance in the proportion of men to women in the present study. In addition, there were no differences in GOLPH3 expression according to sex. Further studies are needed to explore the contribution of sex to prognosis in patients with early stage PTC.

Previous studies [26-28] have suggested that tumor size is associated with relapse after PTC surgery. In the present study, there was no dif-

ference in GOLPH3 expression between patients with T1 and T2 tumors, but capsule integrity and nodular infiltration or invasion were closely associated with postsurgical relapse. Therefore, it appears that the integrity of the thyroid nodular capsule might be a key risk factor for relapse.

In addition, GOLPH3 expression was significantly associated with thyroid nodular capsule integrity suggesting that both capsule integrity and GOLPH3 expression represent significant independent factors associated with PTC prognosis.

Postsurgical relapse in patients with PTC has been associated with the surgical method used [29] and the presence of residual papillary thyroid after the first surgery [30]. For the patients with single lobular PTC without lymph node metastasis, unilateral lobe resection is the optimal surgical treatment. However, if intraoperative analysis or presurgical analysis reveals positivity in the VI region, and to some extent the III and IV regions, then neck lymph node dissection or isotope treatment after surgery is strongly recommended. Congruent with these suggestions, the present study indicated that the positive neck lymph nodes in the VI region or the unilateral neck nodes increased the postsurgical relapse rate. Furthermore, GOLPH3 expression was significantly associated with lymph node status, and both were significant independent prognosticators, which suggest that GOLPH3 expression could be used as a biomarker for lymph node status.

While the prognosis of PTC is good and subsequent surgery or radioisotope therapy do not affect survival, they can have a significant impact on patient quality of life. Therefore, it is critical to evaluate patient condition prior to the first surgery. The association of GOLPH3 expression with relapse was comparable to the associations of relapse with capsule invasion and lymph node metastasis. Although the mechanism of GOLPH3 function in PTC is not clear, overexpression of GOLPH3 is a valuable indicator for postsurgical prognosis and would be a useful biomarker to screen high-risk patients prior to the initial surgery.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Qing-An Zeng, Department of General Surgery, The Fifth Affiliated Hospital of Sun Yat-sen University, 52 Meihua Dong Road, Zhuhai 519000, Guangdong, P. R. China. Tel: +86-756-2528862; Fax: +86-756-2528308; E-mail: 2528894435@qq.com

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