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Clinical Significance of TACC1 and TFF3 Expression in Gastric Carcinoma*

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ABSTRACT Objective: To examined the expression and clinical significance of transforming acidic coiled-coil 1 (TACC1) and Trefoil factor 3 (TFF3) in human gastric carcinoma. **Methods:** TACC1 and TFF3 expression was detected by immunohistochemical staining. Relationship between TACC1 and TFF3 expression and clinicopathologic parameters, and their prognostic values were analyzed by x^2 test, Kaplan-Meier method, and Cox uni- and multivariate survival models. **Results:** TACC1and TFF3 expression rate was higher in the advanced gastric cancer, recurrent cancer and patients with lymph node metastasis. In addition, TFF3 has higher expression in gastric cancer patients with larger size (Tumor size>4 cm). Age, lymph node metastasis,TACC1 and TFF3 expression were significantly associated with low survival ($P<0.05$). The result of multivariate analysis showed that TACC1 and TFF3 expression were independent prognostic predictors. **Conclusions:** TACC1 and TFF3 expression was an independent predictor of short survival in gastric carcinoma. Patients with both TACC1and TFF3 expressions have the shortest survival than other groups.

Key words: TACC1; TFF3; Gastric cancer; Prognosis

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Introduction

From a global perspective, gastric carcinoma is the third most common malignant tumor and the second most common cause of cancer-related death[1,2]. Despite the recent progresses in the development of new therapeutic strategies and in early diagnosis, the prognosis of gastric carcinoma continues to be poor, with <20 % of patients surviving at 5 years [3]. With these perspectives, it becomes of paramount importance to identify factors helping to predict survival and/or response to treatment, to choose better among the available therapeutic tools. In addition to the proven prognostic indicators (age, sex, Laurens' histology, and margins), a series of molecular markers are currently under investigation as survival predictors[4-6].

Transforming acidic coiled coil 1 (TACC1) was the founding member of a novel and expanding family of genes which encode a C-terminal coiled coil domain. Still identified TACC1 while investigating the region at 8p11 that is amplified in 10-15 % of breast tumor samples[7]. SAGE (Serial Analysis of Gene expression) analysis has suggests that TACC1 is down-regulated in ovarian tumors and ovarian cancer cell lines [8]. In addition, in a previous study, which searched for immunogenic proteins in gastric cancer, which resulted in the identification of 14 antigens, including TACC1 [9]. Already, there is evidence for its roles in hematopoiesis, cell division and neoplasia.

Trefoil factor 3 (TFF3) is a member of the trefoil factor family (TFF) peptides. It is conserved among species and has trefoil domain and C-terminal dimerization domain [10]. It was secreted by goblet cells and specifically localized to the surface of intestinal mucosa mainly in the diffuse-type gastric cancer cells[11]. TFF3 has a higher expression level in gastric biopsies with intestinal metaplasia, and a progressive increase in TFF3 expression was also seen from non-neoplastic gastric mucosa to gastric cancer[12]. Various functional aspects of TFF3 have been reported, such as acting as an inflammatory modulator, sustaining mucosa integrity, inhibiting apoptosis via NF-κB pathway, and promoting cell invasion through modulation of E-cadherin/catenin complex function [13,14]. Yamachika reported that TACC1 and TFF3 were downstream genes of HER2/neu, which was found to be changed by HER-2 status[15]. The aim of the work presented in this report was to investigate the expression pattern and clinical implications of TACC1 and TFF3 in gastric carcinoma.

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1 Materials and Methods

1.1 Patients and tissue samples

112 patients with gastric cancer, who received surgical resection treatment without prior neoadjuvant treatment in the Affiliated Hospital of Medical College Qingdao University, were enrolled in the study from Nov. 2007 to Mar. 2009 with approval of the ethics committee of our center. The median follow-up time for all patients was 38 months (range 3 to 54months). All specimens were collected from the patients with informed consents.

1.2 Immunohistochemistry (IHC)

IHC was performed according to standard protocols. Briefly, the paraffin-embedded tissue blocks were sectioned in 4 μm slides. After dewaxing and hydration, the slides were rinsed in PBS and blocked endogenous peroxidase activity with 3% hydrogen peroxide for 10 minutes. Antigen retrieval for 10 minutes later, the specimens were incubated with rabbit polyclonal to TACC1 and mouse monoclonal antibody against TFF3 at 37 ℃ for 90 minutes, using PBS instead of antibody served as negative control. After washed, the sections incubated with the secondary antibodies for 60 min at 37℃ in a humid chamber. Then the sections were washed 3 × 3 min with PBS, followed by the addition of diaminobenzidine (DAB) as a visualization, and hematoxylin for counterstain.

1.3 Evaluation of staining

Slides were first scanned at × 100 magnifications, and then three cellular areas were selected and evaluated at × 400 magnifications. Immunohistochemical staining was assessed semiquantitatively by detecting both the extent of staining (0, 0 %; 1, 0-10 %; 2, 10-50 %; 3, 50-80 %；4，80-100 %) and intensity of the staining (0 for nonstaining, 1 for yellow staining, 2 for brown yellows staining, and 3 for browns taining, respectively). The weighted score for each case equivalent to the intensity and extent of staining were multiplied. The weighted scores of 0 to 4were considered negative and 5 to 12 were positive.

1.4 Statistical analysis

All statistical analyses were performed by using SAS 9.2 software. Associations between TACC1 and TFF3 expression and clinicopathological variables were analyzed by using x^2 test and Fisher's Exact test. Survival curves were estimated using the Univariate Cox analysis, and differences between the groups were compared using the log-rank test. For multivariate analysis, prognostic factors were analyzed using Cox's proportional hazard model. A P-value less than 0.05 was considered statistically significant.

2 Results

2.1 The expression of TACC1 and TFF3 in gastric cancer tissues

The distribution pattern of expression of the TACC1 and TFF3 were located in the cytoplasm of the cancer cells as shown in Fig 1 .The expression of TACC1 in 57 (51%) cases of human gastric cancers, with TFF3 observed in 50% (56/112) of tumors. TACC1 negative (a) and positive (b) expressions and TFF3 negative (c) and positive (d) expressions in gastric cancer tissue were detected by the immunohistochemical staining(Fig.1).

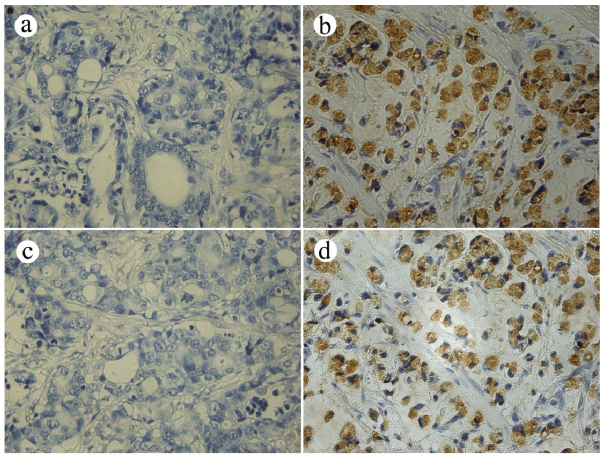

Fig. 1 The expression of TACC1 and TFF3 detected by immunohistochemical staining in gastric cancer patients' tissue samples

2.2 The positive of TACC1 and TFF3 immunohistochemical staining correlates with clinicopathological characteristics

TACC1and TFF3 expression rate was higher in the advanced gastric cancer(TACC1, 57% vs. 43%; TFF3, 56% vs. 44%), recurrent cancer (TACC1, 74% vs. 26%; TFF3, 67% vs. 33%) and patients with lymph node metastasis (TACC1, 70% vs. 30%; TFF3, 60% vs. 40%) than that in early gastric cancer, no recurrence or no lymph node metastasis patients (P<0.05). Only TFF3 expression was significantly higher in patients with larger size (66% vs.34%; P<0.05).

2.3 Correlation between TACC1, TFF3 expression and patient survival

Univariate analysis indicated that clinical variables, including age (P=0.0289), Lymph node metastasis (P<0.0001), TACC1 expression (P=0.0047), TFF3 expression (P=0.0373) and co-expression of TACC1 and TFF3 (P=0.0004) were significantly associated with a short survival time. Patients with positive TACC1/TFF3 staining had a much poorer prognosis than those with negative TACC1/TFF3staining. Both TACC1 and TFF3 expressions had the shortest survival(Fig.2). Furthermore, multivariate Cox regression analyses were performed to evaluate that TACC1 and TFF3 expression in gastric cancer patients was an independent prognostic factor for adverse outcome(Table 1).

Survival analyses with the different TACC1expression a, TFF3 expression b and co-expression c were shown by Log-Rank survival curves. The positive expression was shown as a black discontinuous line, and negative one was a black continuous line one in the Fig.2.

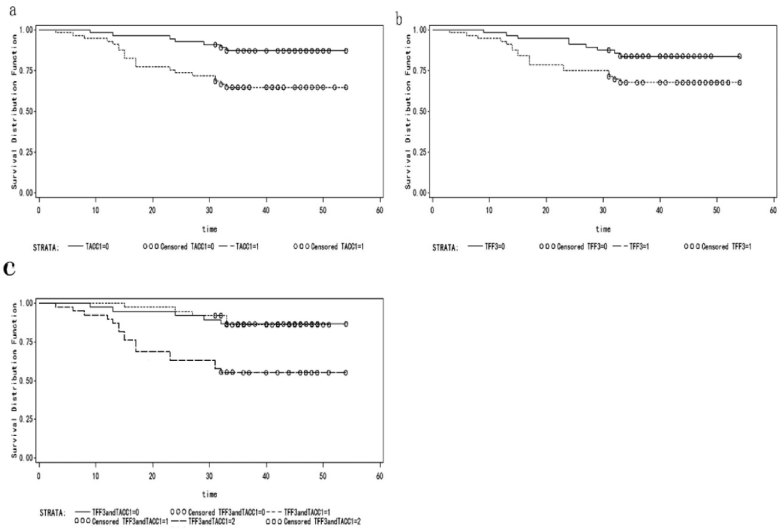

Fig. 2 Survival analyses for gastric cancer patients with TACC1 or TFF3 expression

Table 1 Cox multivariate analysis of prognostic factors of gastric carcinoma

Variables		Number n=112(%)	x^2	P	Hazard Ratio	95%CI
Age	<60	49(44)	4.382	0.0363	2.508	1.06-5.934
	≥ 60	63(56)				
Lymph node metastasis						
	No	49(44)	10.60	0.0011	7.364	2.214-24.49
	Yes	63(56)				
TACC1						
	Negative	55(49)	6.616	0.0101	3.098	1.309-7.331
	Positive	57(51)				
TFF3						
	Negative	56(50)	4.089	0.0432	2.284	1.026-5.088
	Positive	56(50)				

3 Discussion

Given the frequent failure of conventional treatment strategies, many cancer-related molecules have been characterized with the goal of developing novel anticancer therapies, including targeted drugs and antibodies and cancer vaccines[16,17]. Studies have documented that TACC1 expression is thought to be important in the development and progression of several human cancers, such as breast, ovarian tumors and gastric cancer[7,9]. However, few studies have examined the expression and clinical significance of TACC1 in human gastric cancer progression and prognosis. Based on the results of this study, we propose a bold hypothesis that a preoperative determination of TACC1 expression may be useful in predicting the therapeutic effect and postoperative survival of human gastric cancer. The exact mechanisms behind this are still unclear, but it provides a new idea for our further research.

Researchers has shown that induction of TFF3 together with the progressive loss of TFF1 and TFF2 is possibly involved in the early stage of the multi-step gastric cancer pathway[12]. Other study had also reported that over 50 % TFF3 expression was detected in gastric carcinomas [12,15,18]. Previous study from Dhar demonstrated that TFF3 might be a possible role in tumor angiogenesis [8]. Furthermore, Guleng recently have also reported that the mRNA expressions of VEGF and HIF-1a induced by hypoxia is up regulated by overexpression of TFF3 [19]. Although this results were not referred to this in the article，we can suggests that TFF3 may has a oncogenic function either by itself or by cooperating with the other factors in the early stage of the gastric cancer development, hence? to inhibit of TFF3 expression may be a new direction for treatment of gastric cancer.

In the present study, we provide strong evidence that molecular markers of TACC1 and TFF3 are indicators of adverse outcome on survival analysis, independently of classical characteristics. This is similar to our previous research [20]. Patients have the shortest survival with both TACC1 and TFF3 expression, which is accordant with the hypothesis that both TACC1 and TFF3 expression level might be closely correlated with up-regulated migration and high invasiveness and poor prognosis of gastric carcinoma. Hence we can choose the TACC1 and TFF3 as a target molecule or selective marker, which may result in effective therapy for gas-

tric cancer in the clinical.

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TACC1 及 TFF3 在胃癌组织中的表达及其临床意义 *

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摘要 目的：探讨转化酸性卷曲螺旋蛋白 1(TACC1)和肠三叶因子(TFF3)在胃癌组织中的表达及其临床意义。**方法：**采用免疫组化方法检测胃癌组织中 TACC1 及 TFF3 的表达情况。采用 x^2 检验、乘积极限法及单因素、多因素生存分析等统计学方法，分析胃癌组织中 TACC1 及 TFF3 的表达与患者临床病理参数及预后的关系。**结果：**TACC1 和 TFF3 在进展期胃癌、有淋巴结转移及复发的胃癌组织中的表达率较高。此外，TFF3 在较大肿瘤(肿瘤大小 >4 cm)的胃癌组织中的表达较高。单因素生存分析显示年龄、淋巴结转移、TACC1 和 TFF3 表达与低生存期显著相关($P<0.05$)。多因素分析结果表明，TACC1 和 TFF3 的表达是独立的预后预测因子。**结论：**TACC1 和 TFF3 的表达可以作为胃癌的独立不良预后因子，而且在胃癌组织中 TACC1 和 TFF3 共同表达的患者生存时间更短。

关键词：ACC1；TFF3；胃癌；预后

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