

# Prognostic Significance of TACC1 Expression in Gastric Carcinoma

<sup>1</sup> Qian Zhang, <sup>1</sup> Jing Lv, <sup>2</sup> Yu Li, <sup>1</sup> Kewei Liu, <sup>1</sup> Weiwei Qi, <sup>1</sup> Wensheng Qiu\*

<sup>1</sup>Department of Oncology, Affiliated Hospital of Qingdao University, 16 Jiangsu Road, Qingdao 266003, Shandong, China

<sup>2</sup>Department of General Surgery, Affiliated Hospital of Qingdao University, 16 Jiangsu Road, Qingdao 266003, Shandong, China

---

## Article Information

### Article history:

Received 12 November 2013

Revised 13 December 2013

Accepted 1 January 2014

Available online 31 January 2014

---

### Keywords:

TACC1

Gastric cancer

Prognosis

Progression

---

## Abstract

TACC1 protein belongs to the transforming acidic coiled-coil family shown to interact with the gastric cancer. In the present study we analyzed the expression of TACC1 in gastric cancer by means of immunohistochemistry, and verified its prognostic significance by univariate and multivariate Cox analyses. In addition, the correlation between TACC1 expression and clinicopathological factors was tested by chi-square test and Fisher's exact test. The results demonstrated that TACC1 expression was linked to gastric wall invasion (T), lymph node metastasis (N), tumor node metastasis (TNM) stage and likelihood of recurrence ( $p < 0.05$ ). The Cox multivariate analysis indicated that the TACC1 expression was an independent indicator for shorter survival ( $p = 0.048$ ). In conclusion, the study suggested that TACC1 is a favorable and independent prognostic indicator for gastric cancer.

---

## 1. Introduction

Gastric cancer is one of the most common cancers, accounting for approximately 17.2 % of all malignant tumors. Gastric cancer is characterized by invasion and metastasis, which

mainly contributes to the lethality of this disease [1, 2]. The molecular mechanisms underlying these processes involve changes in the expression of various genes and research in this area is becoming increasingly popular. A major focus in the field of clinical gastric cancer research has

---

\* Corresponding author: [qiuws133@126.com](mailto:qiuws133@126.com)

been to identify biological markers associated with these processes.

TACC1 (transforming acidic coiled-coil 1) [3] belongs to a family of three paralogous [4] genes that also includes TACC2 [5] and TACC3 [6]. Transforming acidic coiled-coil (TACC) proteins emerged initially as a group of proteins implicated in cancer. As their family name implies, changes in their expression have been associated with cell transformation, with each of the three human members of the family (TACC1-3) localized to genomic regions amplified in some cancers, and with changes in TACC expression noted even in tumors lacking obvious DNA rearrangements. The first member of the TACC family to be discovered was identified in a search of genomic regions that are amplified in breast cancer. TACC1 could promote cellular transformation because of its highly acidic nature and the presence of a predicted coiled-coil domain at its C terminus (now known as the TACC domain) [3]. Some reports have shown that the TACC1 gene was originally identified within the 8p11 human breast cancer amplicon, and its over-expression has been shown to cause cellular transformation and to promote mammary tumorigenesis [3, 7]. However, no major correlation has emerged to date between the significance of TACC1 expression in the gastric cancer and prognosis. The present study was undertaken to analyze the clinical features of TACC1 expression in gastric cancer and to determine their clinical implications.

## 2. Materials and Methods

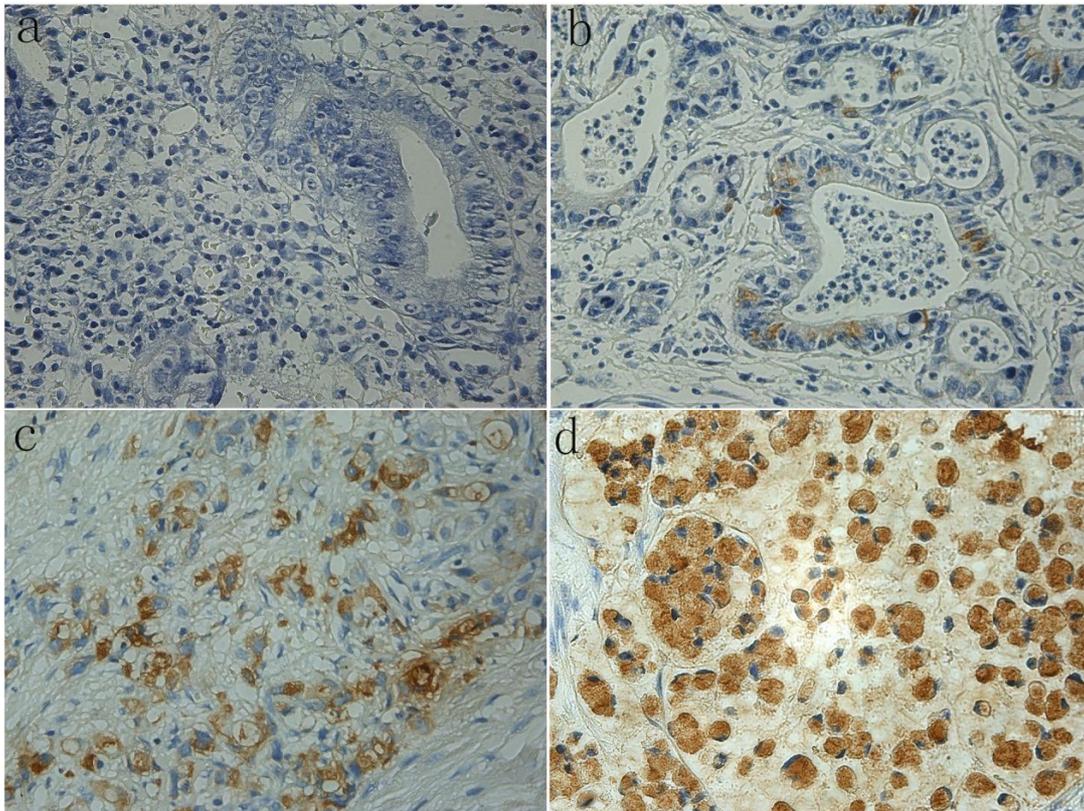
### 2.1 Patients and Tissue samples

Tissue samples were collected from 130 gastric cancer patients (87 male, 43 female; median age 58 years, range 26–90 years) who were enrolled with approval of the ethics committee of our Center and who had received surgical resection from the Department of Surgery, Affiliated Hospital of Qingdao University between November, 2007 and March, 2009. None of the patients were treated with

radiotherapy or perioperative chemotherapy. Resected tissues had been formalin-fixed, paraffin-embedded and diagnosed clinically and histopathologically at the Departments of Gastrointestinal Surgery and Pathology before use. For all patients, the median follow-up time was 40 months (range 3 to 56 months). The survival time was calculated from the date of surgery to the follow-up dead-line or date of death, which was predominantly due to carcinoma recurrence or metastasis. The study items included age, gender, tumor size, gastric wall invasion, lymph node metastasis, histological differentiation, TNM stage and recurrence. And all patients have signed a letter of informed consent.

### 2.2 Immunohistochemistry

Immunohistochemical (IHC) analysis was performed to study TACC1 expression in 130 human gastric cancers. The paraffin-embedded tissue blocks were sectioned in 4  $\mu$ m slices and deparaffinized in 100 % xylene and rehydrated in descending ethanol series according to standard protocols. Heat-induced antigen retrieval was performed in 10 mM citrate buffer at 100 °C for 2 min. Endogenous peroxidase activity and nonspecific antigen were blocked with a peroxidase-blocking reagent containing 3 % hydrogen peroxide and serum. This was followed by incubation with rabbit polyclonal to TACC1 (NBP1-01031, Novus Biologicals, Littleton, USA) for 90 minutes, using PBS instead of antibody as a negative control. After washing, the sections were incubated with 1 and 2 reagent of PV9005 mouse hypersensitivity in two-step immunohistochemical Kit (Beijing fir Jinqiao) for a total of 60 minutes at 37°C in a humid chamber. Then the sections were washed for 3 $\times$ 3-min with PBS, and the peroxidase reaction was developed with 3, 3'-diaminobenzidine (DAB) chromogen solution in DAB buffer substrate. The sections were visualized with DAB, counterstained with hematoxylin, mounted in neutral gum, and analyzed using a bright field microscope.



**Fig1.** The expression of TACC1 in gastric carcinoma tissues by immunohistochemical method. Patients with a score of “-/+” were classified as the negative expression group (a and b), whereas those with “+++ /+++” were classified as the positive expression group (c and d). Magnification 400 ×

### 2.3 Evaluation of Results

TACC1 was stained as buffy colored in the cytoplasm. Scoring was performed according to the extent of staining (0=0%; 1=0-10%; 2=10-50%; 3=50-100%) and intensity of the staining (0 for none, 1 for yellow staining, 2 for brown yellow staining, and 3 for brown staining, respectively). The final score was the product of the two former values (0=-, 1-4 = +, 5-8 = ++, 9-12 = +++). Patients with a score of “-/+” were classified as the negative expression group, whereas those with “+++ /+++” were classified as the positive expression group.

### 2.4 Statistical analysis

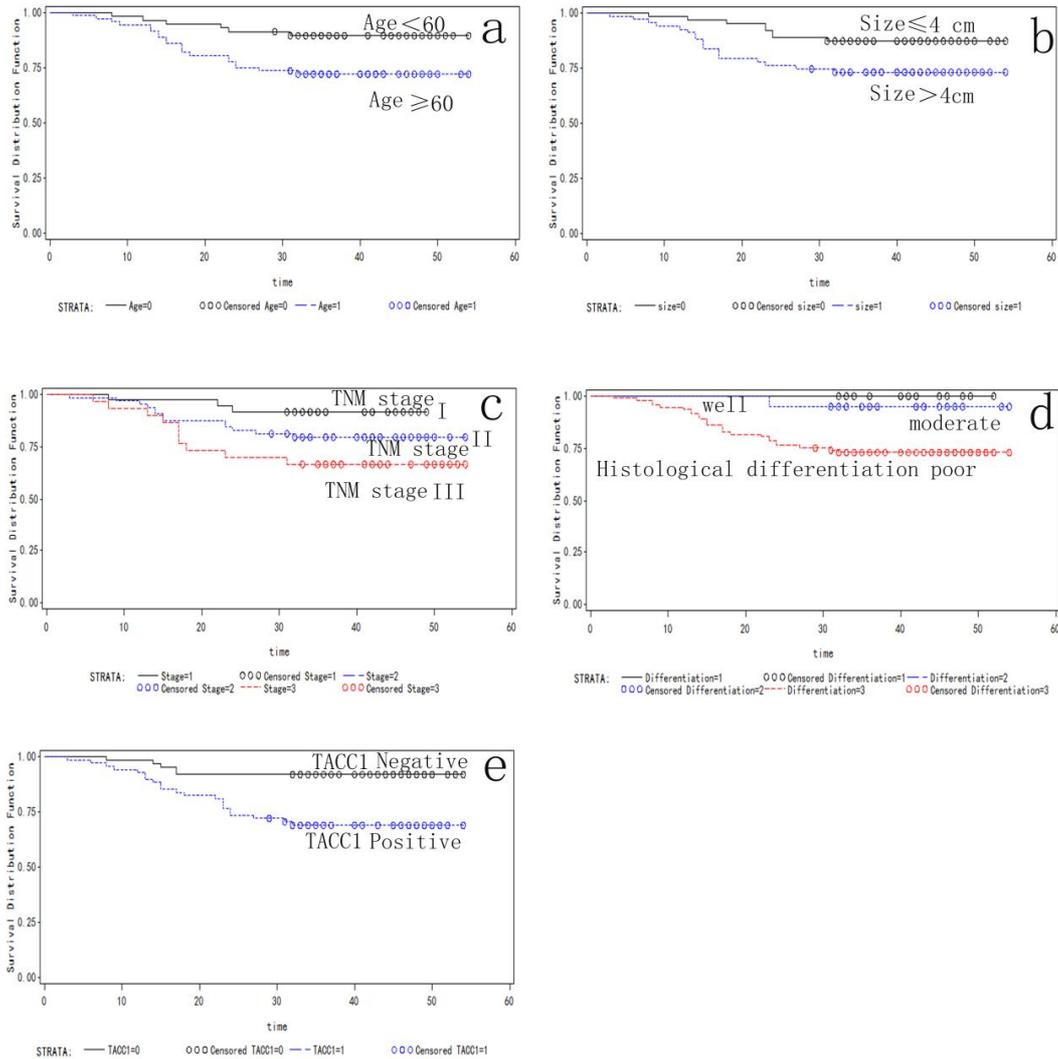
All statistical analyses were performed using

SAS 9.2 software. Correlation between TACC1 expression and clinicopathological variables was estimated using  $\chi^2$  or Fisher exact tests. Survival curves were estimated using the Univariate Cox analysis, and the log-rank test was used to compute differences between the curves. Multivariate analysis using the Cox proportional hazards regression model was performed to assess the prognostic values of the protein expression. In all of the statistical analyses, a  $p < 0.05$  was considered to be statistically significant.

## 3. Result

### 3.1 Immunohistochemical analysis of TACC1 expression in gastric tumor tissues

TACC1 was predominantly localized in the



**Fig2.Survival curves with univariate analyses (log-rank) for age (a), tumor size (b), TNM stage(c), histological differentiation (d) and TACC1 expression (e) in all patients with gastric carcinoma. Patients with older age, larger size, higher TNM stage, lower stages of differentiation, and TACC1 expression positive had a significantly worse survival.**

Cytoplasm of the cancer cells. Immunohistochemical analysis revealed a significant increase in the intensity of TACC1 expression and in the percentage of positively stained cells in gastric cancer specimens (Fig.1). Indeed, TACC1 was expressed at moderate (++) or strong (+++) levels in 52 % (68/130) of carcinoma samples (Fig.1 c,d). Patients' demographical, clinical and histopathological variables are reviewed in Table 1.

**3.2 TACC1 expression and clinical features**

**correlations**

Analysis of TACC1 expression with respect to clinicopathological features revealed that positive expression of TACC1 was correlated with gastric wall invasion (T), lymph node metastasis (N), tumor node metastasis (TNM) stage and recurrence (P < 0.05). In contrast, TACC1 expression was not correlated with age, gender, tumor size or histological differentiation (P > 0.05) (Table 1). Moreover, we observed that the TACC1 expression increased robustly in tumors

of higher pathologic T category (T3>T2>T1), higher TNM stage (III>II>I), and with nodal involvement (N1/N2/N3>N0) and postsurgical recurrence. Although we also found that TACC1 levels displayed a trend toward increased expression at lower stages of differentiation, this was not statistically significant ( $P > 0.05$ ) (Table 1).

**Table 1. Correlation between clinical features and TACC1 expression**

Clinicall features	Cases n(%)	TACC1		P-Value
		Negative cases n(%)	Positive cases n(%)	
Number	130	62(48)	68(52)	
Age				
<60	58	26(45)	32(55)	0.55
≥60	72	36(50)	36(55)	
Gender				
Male	87	44(51)	43(49)	0.34
Female	43	18(42)	25(58)	
Tumor size				
≤4 cm	63	31(49)	32(51)	0.73
>4cm	67	31(46)	36(54)	
T(TNM stage)				
T1	14	10(71)	4(29)	0.0035
T2	29	19(66)	10(34)	
T3	54	21(39)	33(61)	
T4	33	12(36)	21(64)	
N(TNM stage)				
N0	51	30(59)	21(41)	0.0033
N1	57	28(49)	29(51)	
N2	13	2(15)	11(85)	
N3	9	2(22)	7(78)	
TNM stage				
I	36	23(64)	13(36)	0.04
II	64	29(45)	35(55)	
III	30	10(33)	20(67)	
Histological differentiation				
well	17	11(65)	6(35)	0.28
moderate	20	10(50)	10(50)	
poor	93	41(44)	52(56)	
Recurrence				
NO	104	57(55)	47(45)	0.0017
Yes	26	5(19)	21(81)	

### 3.3 Patient survival analysis

The survival analysis revealed that age ( $p = 0.014$ ), tumor size ( $p = 0.035$ ), gastric wall

invasion (T) ( $p = 0.024$ ), lymph node metastasis (N) ( $p = 0.0015$ ), TNM stage ( $p = 0.039$ ), histological differentiation ( $p = 0.0094$ ), and TACC1 expression ( $p = 0.0014$ ) were parameters that correlated with poor patient survival (Table 2). For patients with older age, larger size, higher TNM stage, lower stages of differentiation, TACC1 expression had a much poorer prognosis (Fig. 2). The study did not identify any significant correlation between gender and patient survival ( $p = 0.488$ ).

### 3.4 Multivariate analysis

The multivariate analysis considered parameters that included age, TNM stage, tumor size, T (TNM stage), N (TNM stage), histological differentiation and TACC1 expression. The analysis indicated that TACC1 expression was an independent predictor of shorter survival ( $p = 0.0048$ ). Other independent factors that contributed to survival included T (TNM stage), N (TNM stage) and TNM stage ( $p < 0.0001$ ). Moreover, TACC1 expression was a more accurate predictor (Hazard Ratio 3.4, 95%CI: 1.452-7.959) of outcome than the N (TNM stage) (Hazard Ratio 3.215, 95%CI: 2.227-4.643) (Table 3).

## 4. Discussion

Previous studies have shown that tumor development, invasion and metastasis are associated with distinct gene expression profiles [8]. But the correlations between TACC1 expression and clinicopathologic factors and the prognostic implications have not been determined before. Our study provides strong evidence that a molecular marker TACC1 expression is an effective indicator of adverse outcome both on univariate and multivariate survival analysis, independently of common characteristics among gastric cancer patients after curative resection. On the basis of sufficient data gathered, we could suggest that TACC1 may play a major role in the development of gastric cancer.

**Table2. Cox univariate analysis of initial variables**

characteristics	Number of patients n=130 (%)	$\chi^2$	P- Value
Age			
<60	58(45)	6.004	0.014
≥60	72(55)		
Gender		0.4796	0.488
Male	87(67)		
Female	43(33)		
Tumor size		4.4391	0.035
≤4 cm	63(48)		
>4cm	67(52)		
T(TNM stage)		9.3802	0.024
T1	14(11)		
T2	29(22)		
T3	54(42)		
T4	33(25)		
N(TNM stage)		15.3714	0.0015
N0	51(39)		
N1	57(44)		
N2	13(10)		
N3	9(7)		
TNM stage		6.4560	0.039
I	36(28)		
II	64(49)		
III	30(23)		
Histological differentiation		9.3286	0.0094
well	17(13)		
moderate	20(15)		
poor	93(72)		
TACC1		10.2281	0.0014
Negative	62 (48)		
Positive	68 (52)		

TACC1 is a member of transforming acidic coiled-coil (TACC) protein family [3]. All three currently known human TACC proteins (designated TACC1–3) are centrosome and microtubule binding proteins and they are essential for mitotic spindle formation and function [9, 10] Initial analysis has suggested that TACC1 mRNA levels are expressed at high levels during embryogenesis and are then down regulated as differentiation proceeds, finally being expressed either at low levels or only in restricted tissues [3]. As earlier studies demonstrate the TACC1 gene was originally identified within the 8p11 human breast cancer amplicon, and it is encoded by 18 exons spread

over approximately 150 kb of the proximal short arm of chromosome 8 [11]. Alternative splicing, together with differential promoter usage, results in at least TACC1 transcripts predicted to have significantly different cellular functions based upon the inclusion and exclusion of distinct protein binding motifs [12]. This alternative splicing is particularly important in cancer biology where specific TACC1 splice variants are associated with the malignant phenotype. In a previous study, we searched for immunogenic proteins in gastric cancer by SEREX (serological analysis of recombinant cDNA expression library), and discovered the presence of 14 antigens, including TACC1 [13]. And in the present study, we found that the expression rate of TACC1 in gastric cancer cells exhibited a high level close to 52% (68/130). Based on these reports and our data, we could reasonably suspect that an increase in TACC1 expression may contribute to the occurrence and development of gastric carcinoma. This result shares a similar conclusion with a previous result that the TACC1 protein may be important during tumorigenesis because TACC proteins are also involved in cell cycle checkpoints [14] and can form multiple complexes [15]. Furthermore, some studies showed that its expression was closely related to the development of ovarian and breast cancer [16, 17]. Recent evidence suggests a prognostic value for TACC1 in breast cancer where it associates with endocrine therapy resistance [18].

In our experiment, we also found that the expression of TACC1 was significantly associated with gastric wall invasion (T), lymph node metastasis (N), TNM stage and recurrence ( $P < 0.05$ ) in gastric cancer. The deeper invasion and higher TNM stage, with lymph node metastasis or recurrence group, exhibited a higher expression rate of TACC1. From the above we can draw a conclusion that the TACC1 may play an important role in the progression of gastric carcinoma. Some previous studies prove that TACC1 protein can affect multiple cellular

**Table3. Cox multivariate analysis of prognostic factors**

Characteristics	$\chi^2$	P-Value	Hazard Ratio	95%CI
Age	0.0095	0.9225	0.962	0.445-2.081
Tumor size	3.1647	0.0752	2.068	0.929-4.604
T(TNM stage)	19.8927	<0.0001	4.207	2.238-7.910
N(TNM stage)	38.8381	<0.0001	3.215	2.227-4.643
TNM stage	29.3543	<0.0001	53.459	12.674-225.498
Histological differentiation	2.7367	0.0981	4.607	0.754-28.144
TACC1	7.9519	0.0048	3.4	1.452-7.959

functions, and alterations in its expression are held responsible for cell malignant transformation [12]. And additional studies [19] have reported that some of the interactions of TACCs are with the FHL (four and a half LIM domain ) family of proteins and thus participate in the crosstalk between cell signaling pathways important for cancer development and tumor progression, which is in concordance with our results. Based on these reports, it is reasonable to say that the TACC1 expression is related to development of gastric cancer.

In addition, the survival analysis indicated that the expression of TACC1 was correlated with poor patient survival for gastric carcinoma. We also found that classical characteristics such as age, tumor size, gastric wall invasion, lymph node metastasis, TNM stage and histological differentiation predicted a shorter postsurgical survival, while we further concluded that TACC1 expression was an independent prognostic factor and more powerful predictor of terminal outcomes than the N (TNM stage), which have not previously been determined. In other words, the expression of TACC1 in patients with gastric cancer may be predictive for their outcomes, and provide a new direction for treatment of gastric cancer by inhibiting the molecular marker expression in clinical work.

The exact mechanism of TACC1 in carcinogenesis and tumor progression has not yet been determined. However, research has indicated that TACC1 is able to bind the Sm-like7(LSM7)and SmG proteins involved in telomere formation, while the Transferrin Receptor 2(TFR2) protein, a component of the telomeric complex shelterin, has been observed in a complex with Aurora-C [20, 21]. Thus, it

may be speculated that alterations in TACC1 and/or Aurora-C expression or function may contribute to cancer progression by affecting telomere stability [22, 23]. And several studies have suggested the TACC1 protein level is down-regulated in breast cancer, which may be due to the alteration of the TACC1 function in mRNA metabolism [20]. But so far, the molecular mechanism of the TACC1 in gastric cancer development and/or progression is unclear and it is not within the scope of our study.

In conclusion, our study contributes to a growing body of evidence that TACC1 plays an important role in human gastric cancer development. The expression of the protein may have adverse effect on survival in patients with gastric carcinoma. Given the independent negative prognostic significance of TACC1 expression, the proteins may represent attractive targets for therapeutic inhibition in patients with gastric cancer. Nonetheless, it is known that the biological outcomes of TACC1 are far more complicated than previously anticipated. In the future, the identification of additional factors involved in the activation of TACC1 and signaling pathways will likely provide us with more selective ways to treat the gastric cancer, via targeted inhibition.

### Acknowledgement

The project of medical and health technology development program in Shandong Province (2013WS0260) and Young Scientist Research Award Fund Project of Affiliated Hospital of Qingdao University provide the fund support.

## Conflict of interest statement

We have no conflicts of interest to report.

## References:

- [1] Steeg PS. Tumor metastasis: mechanistic insights and clinical challenges. *Nat Med*,12: 2006 895-904.
- [2] Smith BR, Stabile BE. Extreme aggressiveness and lethality of gastric adenocarcinoma in the very young. *Arch Surg* ,14: 2009 506-510.
- [3] Still IH, Hamilton M, Vince P, Wolfman A, Cowell JK. Cloning of TACC1, an embryonically expressed, potentially transforming coiled coil containing gene, from the 8p11 breast cancer amplicon. *Oncogene*, 18: 1999 4032-4038.
- [4] Popovici C, Leveugle M, Birbaum D, Coulier F. Coparalogy: physical and functional clusterings in the human genome. *Biochem Biophys Res Commun* ,288: 2001 362-370.
- [5] Lauffart B, Gangisetty O, Still IH. Molecular cloning, genomic structure and interactions of the putative breast tumor suppressor TACC2. *Genomics* ,81: 2003 192-201.
- [6] Sadek CM, Pelto-Huikko M, Tujague M, Steffensen KR, Wennerholm M, Gustafsson JA. TACC3 expression is tightly regulated during early differentiation. *Gene Expr Patterns*, 3: 2003 203-211.
- [7] Cully M, Shiu J, Piekorz RP, Muller WJ, Done SJ, Mak TW. Transforming acidic coiled coil 1 promotes transformation and mammary tumorigenesis. *Cancer Res*, 65: 2005 10363-10370.
- [8] Xu ZY, Chen JS, Shu YQ. Gene expression profile towards the prediction of patient survival of gastric cancer. *Biomed Pharmacother*, 64: 2010 133-139.
- [9] Gergely F, Karlsson C, Still I, Cowell J, Kilmartin J, Raff JW. The TACC domain identifies a family of centrosomal proteins that can interact with microtubules. *Proc Natl Acad Sci USA*, 97:2000 14352-14357.
- [10]Gergely F, Kidd D, Jeffers K, Wakefield JG, Raff JW. D-TACC: a novel centrosomal protein required for normal spindle function in the early *Drosophila* embryo. *Embo J* , 19: 2000 241-252.
- [11]Lee MJ, Gergely F, Jeffers K, Peak-Chew SY, Raff JW. Msps/XMAP215 interacts with the centrosomal protein D-TACC to regulate microtubule behaviour. *Nat Cell Biol*, 3: 2001 643-649.
- [12]Still IH, Vettaikorumakankauv AK, DiMatteo A, Liang P. Structure-function evolution of the transforming acidic coiled coil genes revealed by analysis of phylogenetically diverse organisms. *BMC Evol Biol* , 4: 2004 16.
- [13]Line A, Stengrevics A, Slucka Z, Li G, Jankevics E, Rees RC. Serological identification and expression analysis of gastric cancer-associated genes. *Br J Cancer* , 86: 2002 1824-1830.
- [14]Schneider L, Essmann F, Kletke A. The transforming acidic coiled coil-3 protein is essential for spindle-dependent chromosome alignment and mitotic survival. *J Biol Chem*, 282: 2007 29273-29283.
- [15]Lauffart B, Howell SJ, Tasch JE, Cowell JK, Still IH. Interaction of the transforming acidic coiled-coil 1 (TACC1) protein with ch-TOG and GAS41/NuB1 suggests multiple TACC1-containing protein complexes in human cells. *Biochem J*, 363: 2002 195-200.
- [16]Conte N, Delaval B, Ginestier C. TACC1-chTOG-Aurora A protein complex in breast cancer. *Oncogene*, 22: 2003 8102-8116.
- [17]Lauffart B, Vaughan MM, Eddy R. Aberrations of TACC1 and TACC3 are associated with ovarian cancer. *BMC Womens Health*, 5: 2005 8.
- [18]Ghayad SE, Vendrell JA, Bieche I. Identification of TACC1, NOV, and PTTG1 as new candidate genes associated with endocrine therapy resistance in breast cancer. *J Mol Endocrinol*, 42: 2009 87-103.
- [19]Lauffart B, Sondarva GV, Gangisetty O, Cincotta M, Still IH. Interaction of TACC proteins with the FHL family: implications for ERK signaling. *J Cell Commun Signal*, 1: 2007 5-15.
- [20]Conte N, Charafe-Jauffret E, Delaval B. Carcinogenesis and translational controls: TACC1 is down-regulated in human cancers and associates with mRNA regulators. *Oncogene*, 21: 2002 5619-5630.
- [21]Spengler D. The protein kinase Aurora C phosphorylates TRF2. *Cell Cycle*, 6: 2007 2579-2580.
- [22]Campisi J, Kim SH, Lim CS, Rubio M. Cellular senescence, cancer and aging: the telomere connection. *Exp Gerontol* , 36: 2001 1619-1637.
- [23]Campisi J. Cellular senescence as a tumor-suppressor mechanism. *Trends Cell Biol*, 11: 2001 S27-S31.