Int J Clin Exp Pathol 2016;9(2):1810-1818
www.ijcep.com /ISSN:1936-2625/IJCEP0018375

Original Article

Clinical significance of GTSE1 expression in colorectal carcinoma

Tian Tian[1*], Yongxing Wang[2*], Jibin Li[3*], Feng Zhou[4], Jiayue Cao[2], Ziling Wang[1], Xianli He[4], Haichuan Su[5]

[1]College of Life Sciences and Bioengineering, Beijing Jiaotong University, Beijing, China; [2]Department of Respiratory Medicine, 451 Hospital of PLA, Xi'an, China; [3]Experimental Teaching Center of Basic Medicine, Fourth Military Medical University, Xi'an, China; [4]Department of General Surgery, Tangdu Hospital, Fourth Military Medical University, Xi'an, China; [5]Department of Oncology, Tangdu Hospital, Fourth Military Medical University, Xi'an, China. [*]Equal contributors and co-first authors.

Received October 23, 2015; Accepted December 23, 2015; Epub February 1, 2016; Published February 15, 2016

Abstract: GTSE1 is an important cell cycle regulatory gene and located on microtubules, which has been reported to be overexpressed in lung cancer, breast cancer and oral tongue squamous cell carcinoma etc. However, the expression of GTSE1 and its clinical pathological significance remains to be elucidated in colorectal carcinoma (CRC). Public microarray data, TCGA data and immunohistochemical staining were applied to analyze the mRNA or protein expression levels of GTSE1 in CRC. GTSE1 was found to be significantly up-regulated in 198 CRC tissues when compared with those in adjacent non-tumor tissues. Furthermore, our data showed that up-regulation of GTSE1 was associated with age, pathological tumor-node-metastasis (TNM) stage and chemotherapy. Relationship between GTSE1 expression and risk of death or recurrence were assessed by Kaplan-Meier survival curves and Cox regression model, but no notable results were obtained. We presume GTSE1 might act as a potential factor that contributes to progression of CRC.

Keywords: GTSE1, colorectal carcinoma, immunohistochemical staining, tumor-node-metastasis stage, chemotherapy, progression

Introduction

Colorectal carcinoma (CRC) is the third most common malignancies, with over 1 million new cases and approximately 5,00,000 deaths worldwide each year, and the overall 5-year survival is only 25% [1, 2]. In developed countries, high incidence of CRC continues. At the same time, the incidence rate of CRC in developing countries like China has increased dramatically in recent years, especially in big cities. In China, CRC now becomes a significant threat to people's health and is surely a substantial cancer burden. Because of current diagnostic limitations used in the clinical practice, CRC is usually diagnosed at advanced stages. CRC is a comprehensive endpoint deriving from inherited susceptibility, clinical conditions and environmental/life style related risk factors [3-6]. Despite great progress has been achieved in diagnosis and treatment, there remains a lot of tasks in seeking novel biomarkers for early diagnosis and prediction of prognosis in CRC patients.

GTSE1 gene which is located on human chromosome 22 (region q13.2-q13.3) spans about 33 kb on the human genome with 11 introns and 10 exons. Its homolog, Gtse1 gene (once called B99) has been first cloned from a murine cell line and its expression product has been found to be located on microtubule network in a p53 inducible manner. Similarly to the murine Gtse1, human GTSE1 is expressed specifically in G2 and S phase of cell cycle and modulated by p53 and other cell-cycle regulatory mechanisms [7-9]. A series of studies from Schneider C. Group has reported that GTSE1 negatively modulates the trans-activation function of p53 and p53-dependent apoptosis through the physiological interaction between its C-terminal and C-terminal of p53 [10]. Their further study has demonstrated that the ectopic expression of GTSE1 with an intact nuclear export signal

together with functional Mdm2 can enhance the localization of p53 in cytoplasm [11]. GTSE1 shuttles p53 out of the nucleus via PLK1 phosphorylation at Ser435, and regulates the function of p53 during G2 checkpoint recovery [12]. Besides, the N-terminal of GTSE1 is sufficient for p21 stabilization, which is critical for cells cycle control and cellular resistance to microtubule poison paclitaxel [13]. Recent reports have indicated that GTSE1 gathers at growing microtubule plus ends by binding with EB1+TIP, and is required for cell migration especially in breast cancer cell lines. Expression pattern of GTSE1 indicates its potential to increase the invasiveness in breast cancers [14]. Our previous study of GTSE1 in lung cancer has not shown that the over-expression of GTSE1 had a significant association with clinical data [15]. However, the expression and function of GTSE1 in colorectal cancer remains to be elucidated.

In this study, we investigated the expression of GTSE1 using public microarray data, TCGA data and immunohistochemical staining, and analyzed the association of its expression with clinical features in colorectal carcinoma. Thus, we can find out more clues on prognostic value of GTSE1 in colorectal carcinoma.

Materials and methods

Analysis of public microarray and TCGA data

To investigate the mRNA expression level of GTSE1 in colorectal carcinoma, we downloaded four representative public microarray datasets of CRC tissues from GEO database (http://www.ncbi.nlm.nih.gov/geo/), which were used for the comparison between primary tumor and normal counterparts. For each dataset, the ratio of GTSE1 expression level in tumor to normal tissue (log2 transformed) and *P*-value for Student-t test were calculated. Besides, we retrieved the normalized expression data of GTSE1 in CRC patients from RNA-seq data of TCGA (https://tcga-data.nci.nih.gov/tcga/dataAccessMatrix.htm). This data includes 41 pairs of CRC patients samples [16]. Related expression analysis was performed.

Population of colorectal carcinoma patients

A total of 198 CRC patients were recruited immediately after surgery at the Departments of General Surgery in Xijing and Tangdu Hospitals affiliated with the Fourth Military Medical University (Xi'an, China) from July 2006 to Sep 2011. No age, sex, or disease stage restrictions were applied in case recruitment. A pair of colorectal cancer tissues and corresponding adjacent tissues was collected for each patient. The institutional Ethics Committee approval and signed informed consent from each participant for this study have been achieved.

Clinical data collection

We used a standardized epidemiological questionnaire to record all the demographic and personal data including age, sex, ethnicity, residential region, smoking status, alcohol use, education status, body mass index (BMI), and family history of cancer. All clinical data were collected through in-person interviews or medical chart reviews, including time of diagnosis and surgery, time of recurrence and/or death, tumor stage, differentiation, location site, and treatment protocol. A trained clinical specialist performed the standard follow-up through on-site interview, direct calling or medical chart review. The last follow-up data in this study were obtained in Sep 12th 2014, and 10% patients were lost during follow-up.

Immunohistochemical staining

All CRC cancer tissues were assessed by pathologists according to HE staining to assure the suitable rate of tumor cells in the sample. A rabbit anti-hGTSE1 antibody with the Cat. Number bs-2516R (BoAoSen, China) was used. Immunohistochemical staining was performed following the protocol of Histostain-Plus Bulk Kit (invitrogen, USA) as previously described [15].

Evaluation of immunohistochemical staining results

The results of immunohistochemical staining were evaluated for extent and intensity independently by two professional pathologists. Brown staining of nuclear and/or cytoplasm was defined as positive results. According to our previous report [15], the ratio of positive cells was classified into 5 grades: <10% (0), 10-25% (1), 26-50% (2), 51-75% (3), and >75% (4), whereas the intensity was divided into 4 grades: negative (0), light brown (1), brown (2), dark brown (3). For each patient's tissue speci-

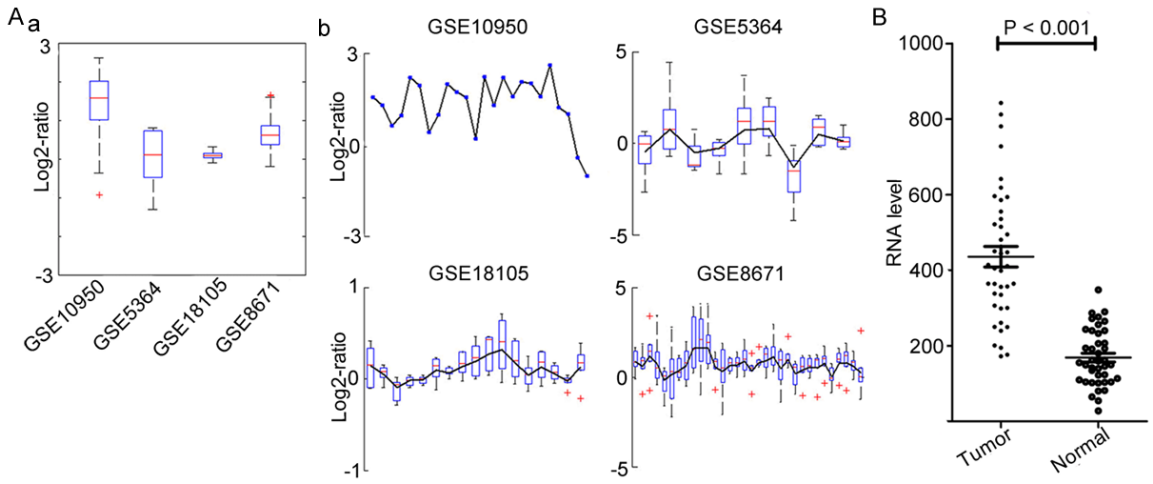

Figure 1. mRNA level of GTSE1 in human colorectal carcinoma tissues. A. GTSE1 mRNA level in four microarray datasets (GSE10950, GSE5364, GSE18105, and GSE8671) of CRC. Fold changes of GTSE1 mRNA level between tumor tissues and non-tumorous tissues for total (a) and each sample (b) were shown. The black line was mean value of the probe replicates in each dataset. The red plus means the value out of range. B. Comparison of GTSE1 mRNA level in 41 pairs of CRC patients from TCGA data (P<0.001).

Table 1. Association of GTSE1 expression level with clinicopathological variables in 198 CRC cases

Variables	Number of patients (%) N=198	GTSE1, mean	*P* value
Age			
≤60	100 (50.5%)	7.54	<0.001
>60	98 (49.5%)	8.66	
Gender			
Male	104 (52.5%)	7.99	0.472
Female	94 (47.5%)	8.22	
Tumor position			
Rectum	108 (54.5%)	8.11	0.939
Colon	90 (45.5%)	8.09	
Tumor stage			
I + II	133 (67.2%)	7.85	0.026
III + IV	65 (32.8%)	8.60	
Tumor differentiation			
Well	30 (15.2%)	7.80	0.419
Moderate + poor	168 (84.4%)	8.15	
Chemotherapy			
Yes	160 (80.8%)	8.25	0.044
No	38 (19.2%)	7.45	
Death			
Yes	60 (30.3%)	8.10	0.984
No	138 (69.7%)	8.10	
Recurrence			
Yes	63 (31.8%)	8.00	0.657
No	135 (68.2%)	8.15	

men, we took pictures of 5 different visual fields under 400× magnification. Then, percentage scores and intensity scores were estimated according to the standards. By multiplying the percentage score and intensity score, total score for each picture was calculated. For each patient, final score was defined as the mean value of five independent visual fields [17, 18].

Statistical analysis

Student's t test and Pearson χ^2 were used to test the association of GTSE1 expression with clinical characteristics, including age, sex, smoking statues, tumor stage, tumor histology, tumor diameter, volume, sub-cellular location, death and recurrence. Hazard ratios (HRs) and 95% confidence interval (95% CI) were estimated with a multivariate Cox proportional hazards model, adjusting to tumor stage where appropriate. Survival chart were plotted by Kaplan-Meier method, and log-rank test were performed to compare the patient overall survival. The SPSS 16.0 statistical package (SPSS, Chicago, IL) was applied in all statistical analysis. A two-sided probability level under 0.05 was defined as statistically significant. The endpoint was overall patient survival or recurrence free survival, which was defined as the time from initial treatment to death or recurrence from any cause [19].

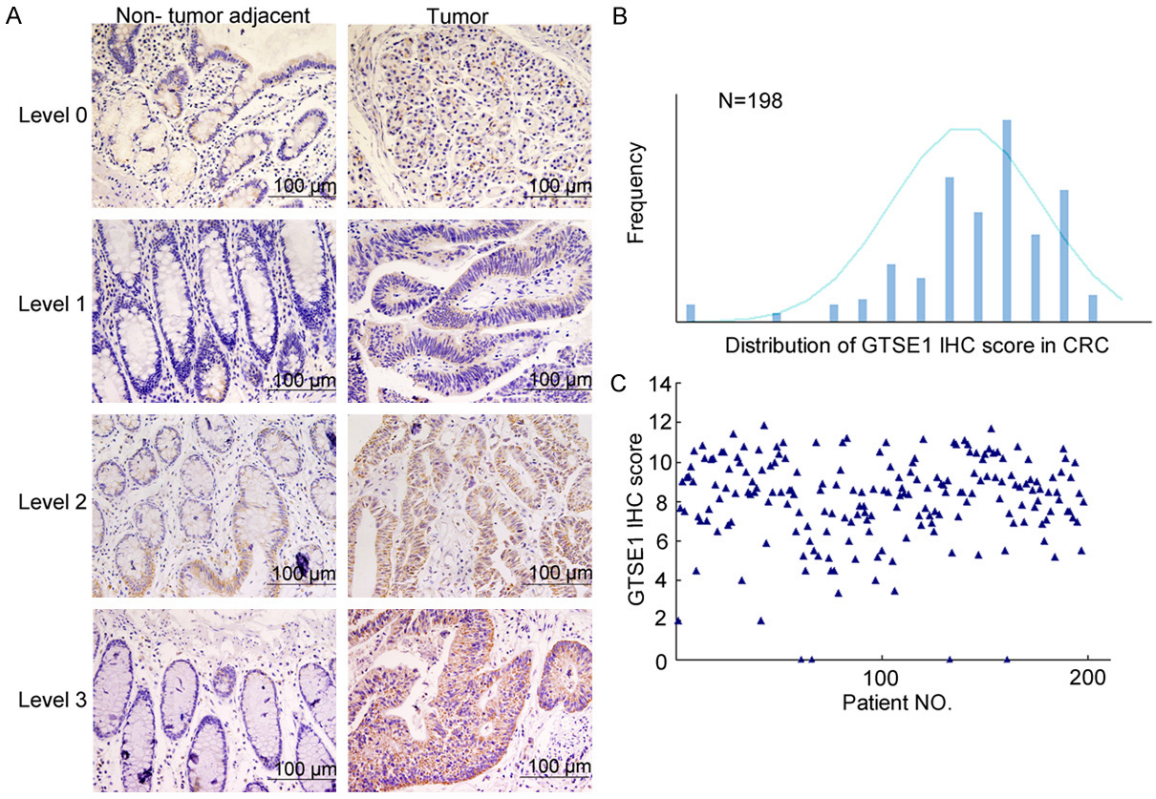

Figure 2. Expression of GTSE1 protein in colorectal cancer tissues by immunohistochemical (IHC) staining and distribution of GTSE1 IHC scores in 198 CRC patients. A. Level 0 to Level 3 samples demonstrate low to high GTSE1 protein expression in colorectal cancer tissues (right) compared to its non-tumor adjacent tissues (left). Magnification, ×400. B. Bar-graph illustrating the frequency and range of GTSE1 IHC staining index of 198 CRC patients. C. The distribution of pathological IHC scores of GTSE1 in 198 CRC patients.

Results

Elevated mRNA expression of GTSE1 in human CRC microarray and TCGA data

We explored the public microarray datasets (colorectal cancer: GSE10950, GSE5364, GSE18105, GSE8671) to check the mRNA level of GTSE1. The $\log_2$ transformed fold change of GTSE1 mRNA level in CRC tissue versus non-tumor tissue was shown in **Figure 1A**. Our results showed that GTSE1 was significantly over-expressed in dataset GSE10950, GSE5364 and GSE8671 with the t-test *p*-value <0.05. Normalized GTSE1 expression files in 41 pairs of CRC patients' samples from TCGA were also retrieved. As demonstrated in **Figure 1B**, GTSE1 mRNA expression was notably higher in CRC tissues compared to corresponding normal tissues with the *p*-value <0.001.

Distribution of patients' characteristics

We summarized the characteristics of CRC patients in **Table 1**. The median age is 60 years old at diagnosis (range from 25 to 88 years old). The median value of biomarker CEA for CRC is 3.73 (range from 0-421, data not shown) [20, 21]. 104 patients (52.5%) were male while the other 94 patients (47.5%) were female. In 90 patients (45.5%), tumor was found in colon. And in other 108 patients (54.5%), tumor was found in rectum. Tumor stage distributions were as following: stage 0/I + stage II (n=133, 67.2%), stage III + stage IV (n=65, 32.8%). The majority (n=168, 84.8%) of patients had moderately or poor differentiated tumors. 60 patients died during the period of follow-up (range from 3 months to 96 months) and 63 patients developed recurrence.

Over-expression of GTSE1 protein in human CRC

We detected GTSE1 expression and subcellular localization in 198 CRC and paratumor tissue specimens by immunohistochemistry. Positive nucleus staining of GTSE1 was found in 9 patients' tissues whereas other 189 patients

Table 2. Distributions of GTSE1 level with clinical characteristics

Variables	Low	Median	High	*P* value
Age				
≤60	39	34	27	0.023
>60	25	34	39	
Gender				
Male	34	38	32	0.593
Female	30	30	34	
Tumor position				
Rectum	35	35	38	0.737
Colon	29	33	28	
Tumor stage				
I + II	48	47	38	0.035
III + IV	16	21	28	
Tumor differentiation				
Well	11	8	11	0.941
Moderate + poor	53	60	55	
Chemotherapy				
Yes	46	55	59	0.011
No	18	13	7	
Death				
Yes	18	21	21	0.649
No	46	47	45	
Recurrence				
Yes	22	20	21	0.759
No	42	48	45	

all show cytoplasm localization of GTSE1. Only 4 CRC specimens show a negative GTSE1 immunohistochemical (IHC) staining whereas GTSE1 was all over-expressed in other 194 specimens of cancer tissues with different expression levels (positive staining rate: 98.0%). All paratumor tissues show weak GTSE1 IHC staining in villus cells. To assess GTSE1 expression more accurately, we clarified the intensity score of the GTSE1 expression into 4 different levels. Level 0 was set for negative staining. Light brown, brown and dark brown staining was set as level 2, 3 and 4 respectively (**Figure 2A**). The IHC scores were calculated by the intensity and percentage of each specimen. We calculated the IHC score as the previously described [15]. The distribution of GTSE1 IHC scores in 198 CRC patients was shown in **Figure 2B** and **2C**.

Association of GTSE1 expression with clinicopathological variables of CRC patients

To explore the possible association between GTSE1 expression and clinicopathological variables of CRC patients, we used two different methods for statistical analysis. First, we divided each variable of whole population into 2 groups, and compared the mean IHC score of GTSE1 in two groups to see if there is significant difference. We observed that GTSE1 expression was correlated with age (P<0.001), tumor stage (P=0.026) and chemotherapy (P=0.044) as shown in **Table 1**.

As indicated in **Table 2**, GTSE1 expression scores were then dichotomized into low, median and high groups with the tertile value 7.46 and 9.1 as the cutoff point. Then, we checked the number of patients in 3 different groups of GTSE1 expression for significant difference. Our data revealed that GTSE1 expression was inversely associated with age (P=0.023), tumor stage (P=0.035) and chemotherapy (P=0.011). This result was consistent with those in **Table 1**. No significant association between GTSE1 expression and gender (P=0.593), tumor position (P=0.737) or tumor differentiation (P= 0.941) were found.

Prognostic significance of GTSE1 in CRC patients

For all 198 patients, the median follow-up period was 45 months. During this period, 60 patients were deceased, and 63 patients developed recurrence. Logistic regression analysis was performed to assess the prognostic significance of GTSE1 in 198 CRC patients. No significant association between death risk and GTSE1 expression levels were observed (P= 0.56, adjusted HR=1.21; 95% CI, 0.72-2.06). Also, no significant association were found between recurrence risk and GTSE1 expression (P=0.96, adjusted HR=1.06; 95% CI, 0.63-1.76). We also did the Kaplan-Meier analysis to estimate the relationship between GTSE1 expression and overall survival (OS) or recurrence-free survival (RFS). But no remarkable difference was observed between OS or RFS of both groups with different GTSE1 expression (As shown in **Figure 3A** and **3B**).

To elucidate the association between GTSE1 over-expression and CRC progression, we further analyzed the correlation of GTSE1 expression with two key factors of CRC, the primary tumor size and the degree of spread to regional lymph nodes (As shown in **Table 3**). GTSE1 expression scores were dichotomized into two groups of low and high expression or three

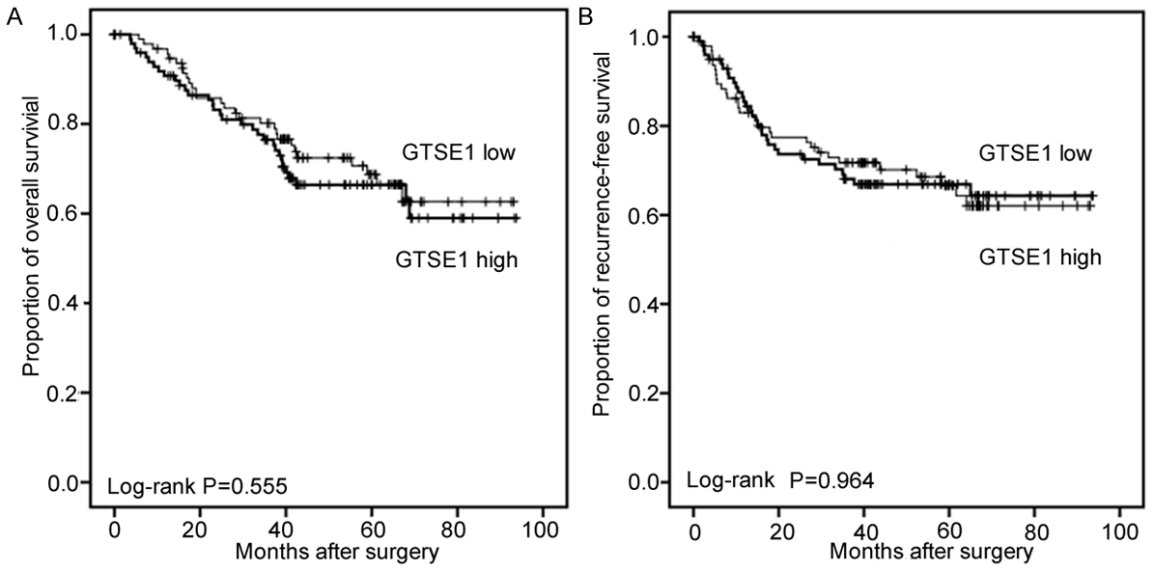

Figure 3. Kaplan-Meier curves of overall survival (OS) and recurrence-free survival (RFS) in CRC patients with low or high GTSE1 expression. A. OS curves: overall survival in CRC patients with high GTSE1 expression and those with low expression (P=0.555). B. RFS curves: recurrence-free survival in CRC patients with high GTSE1 expression and those with low expression (P=0.964).

Table 3. Association of GTSE1 expression with CRC progression

Expression	Tumor stage (T)[a]				Lymph nodes (N)[b]			
	T1, T2 (%)	T3, T4 (%)	OR (95% CI)	P	N0 (%)	N1/2 (%)	OR (95% CI)	P
Two Groups								
GTSE1 low expression	67 (52.3)	29 (41.4)	Ref.		71 (52.6)	25 (39.7)	Ref.	
GTSE1 high expression	61 (47.7)	41 (58.6)	1.55 (0.86-2.80)	0.143	64 (47.4)	38 (60.3)	1.69 (0.92-3.10)	0.092
Three Groups								
GTSE1 low expression	47 (36.7)	17 (24.3)	Ref.		48 (35.6)	16 (25.4)	Ref.	
GTSE1 median expression	40 (31.3)	28 (40.0)	1.94 (0.93-4.04)	0.078	49 (36.3)	19 (30.2)	1.16 (0.54-2.53)	0.702
GTSE1 high expression	41 (32.0)	25 (35.7)	1.69 (0.80-3.55)	0.169	38 (28.1)	28 (44.4)	2.21 (1.05-4.67)	0.037

T size of the primary tumor, N degree of spread to regional lymph nodes, M presence of distant metastasis. [a]Tumour stage T1 + T2 as reference category; T0,Tx and Tis cases were excluded from the analysis. [b]Absence of lymph node affected (N0) as reference category, Nx cases were excluded from the analysis.

Table 4. Modulating effects of chemotherapy on CRC survival by GTSE1 level

GTSE1 level	Death/Total	HR (95% CI)[a]	*P* value	Recurrence/Total	HR (95% CI)[a]	*P* value
In patients with high GTSE1 level						
No Chemotherapy	9/15	Ref.		7/15	Ref.	
Chemotherapy	23/87	0.28 (0.11-0.70)	0.006	25/87	0.40 (0.15-1.04)	0.060
In patients with low GTSE1 level						
No Chemotherapy	7/23	Ref.		7/23	Ref.	
Chemotherapy	21/73	0.69 (0.26-1.86)	0.467	24/73	0.70 (0.27-1.86)	0.477

Abbreviations: CI, confidence interval; HR, hazard ratio. [a]Adjusted by gender, age, TNM stage, differentiation, position.

groups of low, median and high expression. Although GTSE1 expression only showed a trend in two groups analysis, GTSE1 high expression in three groups showed a more significant relationship with degree of spread to regional lymph nodes (P=0.037).

Besides, the modulating effects of GTSE1 expression level on chemotherapy in CRC patients survival were also further investigated. We found that patients who received chemotherapy exhibited a significant protective effect on prognosis in patients with high GTSE1 expression level, but not in those with low GTSE1 expression. When we stratified patients by expression level of GTSE1, we found that patients with high GTSE1 expression benefitted from adjuvant chemotherapy with HRs of 0.28 (P=0.006) for OS and 0.4 for RFS (P=0.06), but those who had low GTSE1 expression did not, with HRs of 0.69 for OS (P=0.467) and 0.70 for RFS (P=0.477) (**Table 4**).

Discussion

GTSE1, which is located on microtubule, has been reported to be up-regulated in lung cancer tissues in our previous study [15]. In breast cancer, over-expressed GTSE1 is associated with invasive potential, tumor stage and time to distant metastasis [14]. In addition, it has been reported that GTSE1 is a potential marker in oral tongue squamous cell carcinoma and its up-regulation correlates with metastasis in gastroenteropancreatic neuroendocrine tumors [22, 23]. Evidences demonstrated GTSE1 is also over-expressed in woman's uterine leiomysoarcoma and metastatic advance Egyptian bladder cancer [24, 25]. Besides, Vinod Vijay Subhash verified that GTSE1 affects cisplatin cytotoxity and is potential for a biomarker in gastric cancer patients [26]. Nevertheless, the potential prognostic value of GTSE1 in colorectal cancer is elusive. In our present study, GTSE1 was found to be up-regulated from evidence of public microarray datasets in colorectal cancer. This finding was further verified in immunohistochemical staining analysis of GTSE1 protein in colorectal cancer tissues when compared with corresponding adjacent tissues. Statistical analysis by two separate ways in 198 colorectal cancer patients showed that over-expression of GTSE1 protein was significantly associated with age and TNM stage. Further investigation showed that high GTSE1 expression is correlated with degree of spread to lymph nodes. For analysis on association of GTSE1 expression with clinicopathological variables, we also included the factors of CEA value and sub-cellular location of GTSE1 expression (nucleus and cytoplasm), but no significant finding was obtained (data not shown). Besides, OS or RFS in CRC patients with different GTSE1 expression levels indicated no significant difference.

New biomarkers in CRC are always in great need in clinical practice. If we can identify a more predictive factor for outcome estimation in CRC, the treatment will be more effective. From our results, high GTSE1 expression has a significant association with older age and advanced TNM stage, suggesting that GTSE1 may contribute to colorectal cancer progression. These results are not similar with our previous study of GTSE1 in lung cancer, in which GTSE1 expression shows no significant association with the clinical variables [15]. Combined with reported results, this may indicate that GTSE1 functions differently in various types of tumors.

Besides, GTSE1 expression exhibited a modulating effect on the efficacy of chemotherapy, indicating that CRC patients with high GTSE1 expression can benefit from adjuvant chemotherapy, which needs to be further validated. This result is consistent with previous study that GTSE1 functions by regulating p21 level to resist paclitaxel, a typical microtubule disrupting drug in chemotherapy [13]. And a very recent research in gastric cancer also implied GTSE1 confers cisplatin resistence [26]. Our finding provides helpful information for the decision-making in individualized chemotherapy of CRC patients. The mechanism underlying our results remains to be elucidated, which is our major aim in future study.

There may be two reasons why GTSE1 up-regulation is associated with CRC progression. First, as former studies reported, GTSE1 is specifically expressed in G2 and S phase in cell cycle and oppositely modulated by p53 and p21, implying that GTSE1 functions to promote cell survival [9, 11, 13]. That is why high GTSE1 expression in CRC cells promotes tumor growth. Second, GTSE1 is also known to be involved in metastasis of some tumor types. GTSE1, orientated on microtubules, can advance the microtubule elongation by gathering at plus end, thus increase the invasive potential in breast cancer [14]. It also helps the cells in resisting paclitaxel [13]. All these evidence suggests that GTSE1 might function in cytoskeleton to induce tumor metastasis. Further investigation in specific

cell lines and animal models is warranted to verify the exact mechanism of GTSE1 in CRC.

In summary, our present study demonstrates that GTSE1 is significantly associated with patient age and TNM stage as well as chemotherapy in colorectal carcinoma. These results provide some evidences that GTSE1 might accelerate tumor progression and serve as a potential indicator or therapeutic targets in colorectal carcinoma. More detailed researches are needed to explore the working mechanism of GTSE1 in CRC.

Acknowledgements

This work was supported by National High Technology Research and Development Programme of China (2014AA021605), grants 31301022, 81272275 and 81372255 from the National Natural Science Foundation of China and Fundamental Research Fund for the Central Universities (2015JBM094).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Xianli He, Department of General Surgery, Tangdu Hospital, Fourth Military Medical University, 169 West Changle Street, Xi'an 710032. Tel: 86-29-84777731; E-mail: wanghe@fmmu.edu.cn; Dr. Haichuan Su, Department of Oncology, Tangdu Hospital, The Fourth Military Medical University, 569 Xinsi Road, Xi'an 710038, China. Tel: 86-29-84778455; Fax: 86-29-84778455; E-mail: suhc@fmmu.edu.cn

References

[1] Center MM, Jemal A, Smith RA, Ward E. Worldwide variations in colorectal cancer. CA Cancer J Clin 2009; 59: 366-78.

[2] Siegel R, Desantis C, Jemal A. Colorectal cancer statistics, 2014. CA Cancer J Clin 2014; 64: 104-17.

[3] Burn J, Mathers J, Bishop DT. Genetics, inheritance and strategies for prevention in populations at high risk of colorectal cancer (CRC). Recent Results Cancer Res 2013; 191: 157-83.

[4] Doubeni CA, Laiyemo AO, Major JM, Schootman M, Lian M, Park Y, Graubard BI, Hollenbeck AR, Sinha R. Socioeconomic status and the risk of colorectal cancer: an analysis of more than a half million adults in the National Institutes of Health-AARP Diet and Health Study. Cancer 2012; 118: 3636-44.

[5] Haggar FA, Boushey RP. Colorectal cancer epidemiology: incidence, mortality, survival, and risk factors. Clin Colon Rectal Surg 2009; 22: 191-7.

[6] Zhang X, Smith-Warner SA, Chan AT, Wu K, Spiegelman D, Fuchs CS, Willett WC, Giovannucci EL. Aspirin use, body mass index, physical activity, plasma C-peptide, and colon cancer risk in US health professionals. Am J Epidemiol 2011; 174: 459-67.

[7] Monte M, Collavin L, Lazarevic D, Utrera R, Dragani TA, Schneider C. Cloning, chromosome mapping and functional characterization of a human homologue of murine gtse-1 (B99) gene. Gene 2000; 254: 229-36.

[8] Utrera R, Collavin L, Lazarevic D, Delia D, Schneider C. A novel p53-inducible gene coding for a microtubule-localized protein with G2-phase-specific expression. EMBO J 1998; 17: 5015-25.

[9] Collavin L, Monte M, Verardo R, Pfleger C, Schneider C. Cell-cycle regulation of the p53-inducible gene B99. FEBS Lett 2000; 481: 57-62.

[10] Monte M, Benetti R, Buscemi G, Sandy P, Del SG, Schneider C. The cell cycle-regulated protein human GTSE-1 controls DNA damage-induced apoptosis by affecting p53 function. J Biol Chem 2003; 278: 30356-64.

[11] Monte M, Benetti R, Collavin L, Marchionni L, Del SG, Schneider C. hGTSE-1 expression stimulates cytoplasmic localization of p53. J Biol Chem 2004; 279: 11744-52.

[12] Liu XS, Li H, Song B, Liu X. Polo-like kinase 1 phosphorylation of G2 and S-phase-expressed 1 protein is essential for p53 inactivation during G2 checkpoint recovery. EMBO Rep 2010; 11: 626-32.

[13] Bublik DR, Scolz M, Triolo G, Monte M, Schneider C. Human GTSE-1 regulates p21 (CIP1/WAF1) stability conferring resistance to paclitaxel treatment. J Biol Chem 2010; 285: 5274-81.

[14] Scolz M, Widlund PO, Piazza S, Bublik DR, Reber S, Peche LY, Ciani Y, Hubner N, Isokane M, Monte M, Ellenberg J, Hyman AA, Schneider C, Bird AW. GTSE1 is a microtubule plus-end tracking protein that regulates EB1-dependent cell migration. PLoS One 2012; 7: e51259.

[15] Tian T, Zhang E, Fei F, Li X, Guo X, Liu B, Li J, Chen Z, Xing J. Up-regulation of GTSE1 lacks a relationship with clinical data in lung cancer. Asian Pac J Cancer Prev 2011; 12: 2039-43.

[16] Jiang L, Zhu W, Streicher K, Morehouse C, Brohawn P, Ge X, Dong Z, Yin X, Zhu G, Gu Y, Ranade K, Higgs BW, Yao Y, Huang J. Increased IR-A/IR-B ratio in non-small cell lung cancers

associates with lower epithelial-mesenchymal transition signature and longer survival in squamous cell lung carcinoma. BMC Cancer 2014; 14: 131.

[17] Ingebrigtsen VA, Boye K, Nesland JM, Nesbakken A, Flatmark K, Fodstad O. B7-H3 expression in colorectal cancer: associations with clinicopathological parameters and patient outcome. BMC Cancer 2014; 14: 602.

[18] Kong LM, Liao CG, Fei F, Guo X, Xing JL, Chen ZN. Transcription factor Sp1 regulates expression of cancer-associated molecule CD147 in human lung cancer. Cancer Sci 2010; 101: 1463-70.

[19] Kim JG, Chae YS, Sohn SK, Cho YY, Moon JH, Park JY, Jeon SW, Lee IT, Choi GS, Jun SH. Vascular endothelial growth factor gene polymorphisms associated with prognosis for patients with colorectal cancer. Clin Cancer Res 2008; 14: 62-6.

[20] Duffy MJ, van DA, Haglund C, Hansson L, Holinski-Feder E, Klapdor R, Lamerz R, Peltomaki P, Sturgeon C, Topolcan O. Tumour markers in colorectal cancer: European Group on Tumour Markers (EGTM) guidelines for clinical use. Eur J Cancer 2007; 43: 1348-60.

[21] Locker GY, Hamilton S, Harris J, Jessup JM, Kemeny N, Macdonald JS, Somerfield MR, Hayes DF, Bast RC Jr. ASCO 2006 update of recommendations for the use of tumor markers in gastrointestinal cancer. J Clin Oncol 2006; 24: 5313-27.

[22] Lee J, Sung CO, Lee EJ, Do IG, Kim HC, Yoon SH, Lee WY, Chun HK, Kim KM, Park YS. Metastasis of neuroendocrine tumors are characterized by increased cell proliferation and reduced expression of the ATM gene. PLoS One 2012; 7: e34456.

[23] Zhou X, Temam S, Oh M, Pungpravat N, Huang BL, Mao L, Wong DT. Global expression-based classification of lymph node metastasis and extracapsular spread of oral tongue squamous cell carcinoma. Neoplasia 2006; 8: 925-32.

[24] Barlin JN, Zhou QC, Leitao MM, Bisogna M, Olvera N, Shih KK, Jacobsen A, Schultz N, Tap WD, Hensley ML, Schwartz GK, Boyd J, Qin LX, Levine DA. Molecular subtypes of uterine leiomyosarcoma and correlation with clinical outcome. Neoplasia 2015; 17: 183-9.

[25] Zekri AR, Hassan ZK, Bahnassy AA, Khaled HM, El-Rouby MN, Haggag RM, bu-Taleb FM. Differentially expressed genes in metastatic advanced egyptian bladder cancer. Asian Pac J Cancer Prev 2015; 16: 3543-9.

[26] Subhash VV, Tan SH, Tan WL, Yeo MS, Xie C, Wong FY, Kiat ZY, Lim R, Yong WP. GTSE1 expression represses apoptotic signaling and confers cisplatin resistance in gastric cancer cells. BMC Cancer 2015; 15: 550.