Clinical Significance of *STC1* Gene Expression in Patients with Colorectal Cancer

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Abstract. *Background: Recent studies suggest that altered patterns of stanniocalcin 1 (STC1) gene expression have a role in human carcinogenesis. This study examined the relationship between the relative expression of the STC1 gene and clinicopathological factors in patients with colorectal cancer. Patients and Methods: Surgical specimens of cancer tissue and adjacent normal mucosa were obtained from 202 patients with colorectal carcinomas. The relative expression levels of STC1 mRNA in the cancer and the normal adjacent mucosa were measured by quantitative real-time, reverse-transcriptase polymerase chain reaction. Results: The relative expression levels of the STC1 gene were higher in the cancer tissue than in the normal adjacent mucosa and high expression of STC1 correlated with poor postoperative survival. Conclusion: High expression of the STC1 gene might be a useful predictor of poor postoperative outcome in patients with colorectal cancer.*

Stanniocalcin (STC) is a glycoprotein hormone that regulates calcium and phosphate levels produced in bony fish by the corpuscle of Stannius, which is located near the kidney (1, 2). A human ortholog of fish STC, STC1, has been identified by molecular biological techniques (3, 4). Human *STC1* complementary DNA (cDNA) encodes a 247 amino-acid protein. The gene resides on the short arm of chromosome 8 (8p11.2-p21) and contains four exons (5, 6). In contrast to fish STC, mammalian STC1 is expressed in various tissues,

including the kidney, ovary, prostate, thyroid, colon, bone and spleen (3, 4, 6), and appears to be involved in not only calcium/phosphate regulation (7-9), but also in diverse biological processes. Modulation of STC1 expression has been demonstrated in numerous developmental, physiological, and pathological processes including pregnancy (10), lactation (10), angiogenesis (11-14), organogenesis (15-18), cerebral ischemia (19), hypertonic stress (20), oxidative stress (21), and apoptosis (22, 23). *STC1* was originally cloned as part of a search for cancer-related genes, and recent studies have indicated that altered STC1 expression patterns may have a role in carcinogenesis. Increased *STC1* gene expression has been found in hepatocellular (24, 25), colorectal (12, 25, 26), and medullary thyroid carcinomas (27), increased STC1 protein expression has been found in ovarian caner (28), and aberrant *STC1* gene expression has been found in breast carcinomas (29-32). The mechanisms involved remain poorly understood and it remains unclear whether the expression of *SCT1* is associated with the malignant potential of cancer. In agreement with other studies, the present results showed that *STC1* gene expression levels were higher in the cancer tissue than in the normal adjacent mucosa. A paralog of *STC1* (*STC2*) was identified by searching expressed sequence tag databases for sequences related to *STC1* (2). *STC2* cDNA has 34% homology with both *STC1* and eel *STC* (2). *STC2* is less strongly related to fish *STC* than to *STC1*. Similar to *STC1*, *STC2* is expressed in various tissues and is associated with several types of cancer, including breast cancer (29), renal cell carcinoma (33), and colorectal cancer (34), but these proteins are thought to have different biological roles (2).

In this study, the expression levels of the *STC1* gene were measured in cancer tissue and adjacent normal mucosa obtained from patients with colorectal cancer. To evaluate the clinical significance of *STC1*, the correlation between the relative expression of this gene and clinicopathological

Table I. *PCR primers and conditions*.

Gene	Primer	Annealing temperature (˚C)	Product size (bp)
STC1	5'-AGGATGATTGCTGAGGTG-3'	60	119
	5'-CTGTTGGAGAAGTGATTGG-3'		
β-Actin	5'-AGTTGCGTTACACCCTTTCTTGAC-3'	60	171
	5'-GCTCGCTCCAACCGACTGC-3'		

features was examined. Additinally, the influence of *STC1* gene expression on the outcomes of patients with colorectal cancer was assessed.

Patients and Methods

Patients and samples. Surgical specimens of cancer tissue and adjacent normal mucosa were obtained from 202 patients with colorectal cancer who underwent surgery at Kanagawa Cancer Center and at the Gastroenterological Center of Yokohama City University Medical Center between 2002 and 2006. Informed consent was obtained from each patient. The ethics committees of Kanagawa Cancer Center and Yokohama City University Medical Center approved the protocol before initiation of the study. All the tissue samples were embedded in Optimal Cutting Temperature (OCT) compound (Sakura Finetechnical Co., Ltd., Tokyo, Japan) and were immediately stored at –80˚C until use. No patient had any other malignancies. The specimens were stained with hematoxylin and eosin and examined histopathologically. Sections that consisted of >80% carcinoma cells were used to prepare the total RNA.

Quantitative real-time, reverse-transcriptase polymerase chain reaction (PCR). The total RNA isolated from the colorectal cancer and adjacent normal mucosa was prepared with the use of Trizol (Gibco Life Tech, Gaithersburg, MD, USA). cDNA was synthesized from 2 μg of total RNA using an iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, CA, USA). After synthesis, the cDNA was diluted 1:4 with water and stored at –20˚C until use. Quantitative real-time PCR was performed with an iQ SYBR-Green Supermix (Bio-Rad Laboratories). The PCR was carried out in a total volume of 15 μl containing cDNA derived from 75 ng of RNA, 0.27 μM of each primer, 7.5 μl of iQ SYBR-Green Supermix containing dATP, dCTP, dGTP and dTTP at concentrations of 400 μM each and 50 units/ml of iTaq DNA polymerase. The PCR consisted of 10 min at 94˚C, followed by 50 cycles of denaturation of the cDNA for 30 s at 94˚C, annealing for 30 s at 60˚C, and a primer extension for 1 min at 72˚C followed by 72˚C for 10 min. The PCR primer sequences of *STC1* and β-actin, used as an internal control, are shown in Table I.

Statistical analysis. The gene expression levels in colorectal cancer were compared with those in normal adjacent mucosa with the use of the Wilcoxon test. The relationships between the gene expression levels and potential explanatory variables, including age, gender, tumor size, histological type, depth of invasion, lymph node metastasis, location, lymphatic invasion, venous invasion and liver metastasis, were evaluated with the chi-square test. The postoperative survival rate was analyzed with the Kaplan-Meier method, and differences in survival rates were assessed with the log-rank test. A Cox proportional-hazards model was used for

multivariate analysis. All the statistical analyses were performed using Dr.SPSS II, version 11.0.1 J for Windows software (SPSS Inc., Chicago, IL, USA). Two-sided *p*-values were calculated, and differences were considered significant at *p*-values of <0.05.

Results

STC1 mRNA expression. *STC1* mRNA expression levels were significantly higher in the cancer tissues than in the normal adjacent mucosa ($p=0.004$; Figure. 1).

Relationship of STC1 gene expression level to clinico-pathological features. The expression levels of the *STC1* gene were categorized as low or high in relation to the median value. The *STC1* gene expression level was not related to age, gender, tumor size, histological type, depth of invasion, lymph node metastasis, tumor location, lymphatic invasion, or venous invasion. However, the *STC1* gene expression level correlated with liver metastasis (low expression: 24/101 [23.8%], high expression: 38/101 [37.6%], $p=0.047$; Table II).

STC1 expression and postoperative survival. Overall survival curves were plotted according to *STC1* mRNA expression level by the Kaplan-Meier method. The median follow-up period was 1178 days. In the study group as a whole (202 patients), the overall survival rate was significantly lower in the patients with high *STC1* mRNA expression than in those with low expression ($p=0.016$; Figure 2).

Univariate analysis with Cox proportional-hazards model identified seven prognostic factors: histological type, tumor size, depth of invasion, lymph node invasion, lymphatic invasion, liver metastasis and *STC1* expression. The other clinicopathological features, such as age, gender, location, and venous invasion, were not statistically significant prognosis factors (Table III). A multivariate analysis of the prognosis factors with a Cox proportional-hazards model confirmed that high *STC1* expression was a significant independent predictor of poor survival in colorectal cancer (Table IV).

Discussion

Wascher *et al*. (32) reported that in early-stage breast cancer, the detection of *STC1* mRNA in bone marrow and blood significantly correlated with multiple histopathological
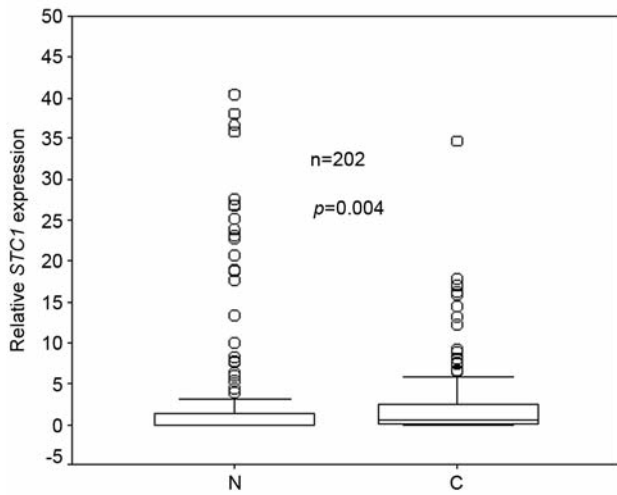
Figure 1. *Comparison of STC1 mRNA expression levels between colorectal cancer tissue (C) and adjacent normal mucosa (N). Box boundaries, the 25th and 75th percentiles of the observed values; capped bars, the 10th and 90th percentiles; solid line, median. p-Value was calculated by the Wilcoxon test. STC1 gene expression levels were higher in cancer tissue than in normal adjacent mucosa (p=0.004).*

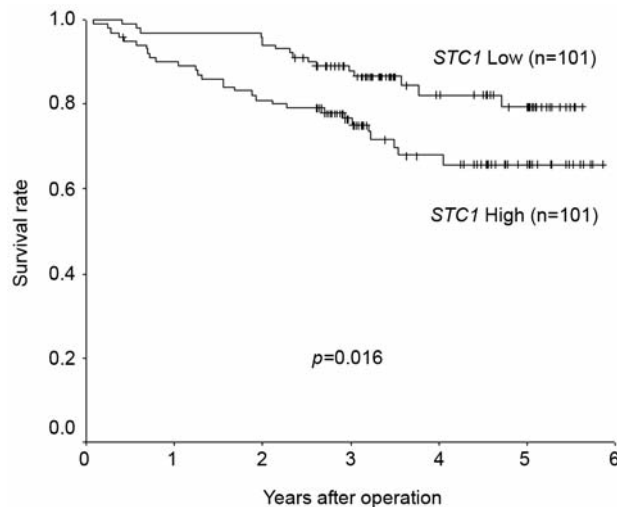Figure 2. *Postoperative survival of patients with colorectal cancer.*

Table II. *Relationship of STC1 gene expression level to clinicopathological features.*

Variable	STC1 expression		*p*-Value
	Low (n=101)	High (n=101)	
Age (years)	66.3±11.1	65.1±10.5	0.432
Gender			
Male	52	59	0.480
Female	49	43	
Tumor size			
≤5 cm	69	62	0.377
>5 cm	32	39	
Histological type			
Well+mod	91	83	0.153
Poor+muc	10	18	
Depth of invasion			
T1, T2	25	25	1.000
T3, T4	76	76	
Lymph node metastasis			
Absent	54	49	0.574
Present	47	52	
Location			
Colon	59	51	0.323
Rectum	42	50	
Lymphatic invasion			
Absent	69	63	0.460
Present	32	38	
Venous invasion			
Absent	44	31	0.080
Present	57	70	
Liver metastasis			
Absent	77	63	0.047
Present	24	38	

n: Number of patients, well: well-differentiated, mod: moderately differentiated, poor: poorly differentiated, muc: mucinous.

prognostic factors, including primary tumor size, number of positive lymph nodes, TMN stage, and overall American Joint Committee on Cancer (AJCC) stage. Gerritsen *et al*. (12) proposed that increased *STC1* expression was related to tumor vasculature in colon cancer. Ieta *et al*. (34) reported that high *STC2* expression positively correlated with lymph node metastasis, lymphatic invasion, tumor depth, tumor size and AJCC stage in colorectal cancer and was associated with significantly poorer overall survival than low *STC2*

expression. However, the clinical significance of *STC1* gene expression in colorectal cancer remains unclear. To our knowledge, no previous study has examined the relationship between *STC1* expression and patient outcome. In the present study, *STC1* expression was associated with liver metastasis.and high *STC1* expression correlated with poor postoperative survival. Fujiwara *et al*. (25) and Wascher *et al*. (32) have suggested that *STC1* mRNA might be a useful molecular marker for the detection of tumor cells in blood. Tumor cells expressing high levels of *STC1* probably exist in the circulation and metastasize *via* the bloodstream, which would be consistent with the finding that a high *STC1* expression level was associated with liver metastasis. In conclusion, high *STC1* gene expression might be a useful predictor of poor postoperative survival in patients with colorectal cancer.

Table III. *Univariate analysis of clinicopathological factors for overall survival.*

Variable	n	Hazard ratio	95% CI	*p*-Value
Age (years)				
≤65	91	1		
>65	111	1.389	0.765-2.525	0.281
Gender				
Male	110	1		
Female	92	0.815	0.451-1.475	0.500
Tumor size				
≤5 cm	131	1		
>5 cm	71	2.379	1.324-4.275	0.004
Histological type				
Well, mod	174	1		
Poor, muc	28	2.410	1.220-4.761	0.011
Depth of invasion				
T1, T2	50	1		
T3, T4	152	16.802	2.314-121.974	0.005
Location				
Colon	110	1		
Rectum	92	1.526	0.847-2.747	0.159
Lymph node metastasis				
Absent	103	1		
Present	99	6.015	2.797-12.993	<0.001
Lymphatic invasion				
Absent	132	1		
Present	70	3.587	1.971-6.526	<0.001
Venous invasion				
Absent	75	1		
Present	127	1.550	0.797-3.015	0.196
Liver metastasis				
Absent	140	1		
Present	62	10.259	5.173-20.347	<0.001
STC1				
Low	101	1		
High	101	2.091	1.135-3.854	0.018

n: Number of patients, CI: confidence interval, well: well-differentiated, mod: moderately differentiated, poor: poorly differentiated, muc: mucinous.

Table IV. *Multivariate analysis of clinicopathological factors for overall survival.*

Variable	Hazard ratio	95% CI	*p*-Value
Tumor size (>5 cm/≤5 cm)	1.467	0.805-2.676	0.211
Histological type (well, mod/poor, muc)	1.247	0.592-2.625	0.561
Depth of invasion (T3, T4/T1, T2)	6.192	0.818-46.853	0.077
Lymph node metastasis (present/absent)	2.774	1.246-6.177	0.012
Lymphatic invasion (present/absent)	1.397	0.708-2.759	0.335
Liver metastasis (present/absent)	5.165	2.510-10.627	<0.001
STC1 (high/low)	1.882	1.014-3.494	0.045

CI: Confidence interval, well: well-differentiated, mod: moderately differentiated, poor: poorly differentiated, muc: mucinous.

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