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Original Article

Expression of guanylyl cyclase C and its prognostic value in different developing stages of colorectal cancer patients

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Abstract: Background: Guanylyl cyclase C (GCC) is a specific and sensitive marker for colorectal cancers, which selectively express on epithelial cells from duodenum to the rectum. Currently the expression of GCC and its prognostic value of different stages of colorectal cancer have not been thoroughly investigated. Methods: Tissue microarray samples of 39 normal mucosa, 47 adenoma and 390 CRC (stage I-IV) tissues were immunohistochemically examined and assayed for GCC expression. Results: GCC were expressed on apical membrane and in cytoplasm. Expression of membranous but not cytoplasmic GCC increased significantly from stage I to stage II CRC (J. Nemenyi test, P=0.006). GCC membranous overexpression was associated with higher TNM (P=0.000), higher T stage (P=0.001) and higher N stage (P=0.000). Kaplan-Meier analyses showed that overexpression of membranous GCC was related to poor overall survival (P=0.025) and disease-free survival (P=0.005). Multivariate analyses showed that higher GCC membranous expression (P=0.014 for OS and P=0.018 for DFS) was independent predictive hazard factors of poor prognosis. Conclusion: GCC membranous expression is a valuable prognostic indicator for colorectal cancer and would be useful in tumor staging especially for stage II tumor diagnosis and treatment selection.

Keywords: Guanylyl cyclase C, biomarker, colorectal cancer, immunohistochemistry, prognosis

Introduction

Colorectal cancer is the third most common cause of cancer-related mortality worldwide [1], resulting in at least 1 million cases and 500,000 deaths annually [2, 3]. At present, treatments for patients with colorectal cancer are determined by clinical stage. Surgery remains potentially curative treatment for the stage I and stage II patients. However, less than 50% of these patients relapse after surgical resection [4]. This occurred because of that surgery removes only detectable tumor. The undetectable micro metastases may cause relapse after operation [5]. Thus, predicting outcome of large amount population of stage II colorectal cancer remains challenging. Currently, numerous specific tumor markers for intestinal epithelium have been characterized by immunohistochemistry or polymerase chain reaction (PCR), also have been used for identification of tumor metastasis and prediction of CRC prognosis [6]. However, few biomarkers are both specific and sensitive for prognosis prediction and therapy selection.

Guanylyl cyclase C (GCC) is a brush border membrane receptor in human beings, which has been shown to be a specific and sensitive marker for colorectal cancers, that selectively expressed by epithelial cells from the duodenum to the rectum, but not by normal esophageal or gastric mucous membrane cells or normal extra mucosal cells [7]. This receptor, bound to both the bacterial heat-stable enterotoxin and the guanylin/uroguanylin peptide ligands, mediates the accumulation of the intracellular cGMP to activate the cyclic nucleotide dependent protein kinases, followed by the phosphorylation of cystic fibrosis trans mem-

brane conductance regulator resulting in the increases of chloride ion efflux and loss of fluid from the cells. In addition, GCC catalyzes a variety of cellular vital processes such as colonic cell proliferation, cell apoptosis and regulation of DNA synthesis [8]. Recently, GCC has been figured to be involved in a novel Rec/PAK/GC/cGMP signaling pathway, providing a new insight in the modulation of cyclizing activity [9]. Certain molecular study demonstrated that GCC mRNA quantified by RT-PCR is overexpressed in CRC tissue [10]. Detection of GCC presence has been measured to be a way of detecting micro metastases to regional lymph nodes in CRCs [11, 12]. To date, the development of GCC expressed in different CRC stages has not been thoroughly investigated.

The focus of this study was to examine the immunohistochemical staining of GCC expression of 476 cases from normal epithelial cells to different stages in developing CRC groups to elucidate the clinical significance of GCC expression in CRC. In addition, GCC expression associated with clinicopathologic parameters and prognosis was also evaluated.

Methods and materials

Patients and samples

Formalin-fixed paraffin embedded tissues from 476 specimens including 39 normal rectal mucosa, 47 colorectal adenomatous polyps, 27 stage I CRC, 140 stage II CRC, 188 stage III CRC and 35 stage IV CRC were collected from the archives of the Department of Pathology at Changhai Hospital of the Second Military Medical University in Shanghai, China. All patients with CRC were diagnosed and operated from December 1999 to December 2009. Inform consent forms were signed from all patients and the research protocol was approved by the Ethics Committee. Histologic sections were reviewed and reclassified based on the current WHO criteria [13].

Tissue microarray (TMA) construction

Corresponding areas of all specimens was identified with HE stained slides from each case and remarked from the paraffin-embedded source blocks. Then the source blocks were cored and 1.5 mm diameter cores were transferred to construct TMA. Each TMA containing 160 cases of normal mucosa to poor-differentiated CRC was assessed.

Immunohistochemistry of GCC

Immunohistochemical staining for GCC (Anti-GUGY2C antibody from Abcam ab122404) was performed on each of the TMAs, using DAB-based staining technique (Dako Chem Mate TM Envision TM Kit, Denmark). 10% neutral buffered formalin was used to fix the paraffin-embedded sections, followed by deparaffinization and rehydration, and then the antigen retrieval was completed with EDTA buffer solution (0.01 mol/L, pH=8.0) by pressure cooker. The slides were incubated with 3% hydrogen peroxides and 4% normal goat serum to confirm that staining reflected specific interaction with GCC, followed by primary antibody (1:550) for 120 minutes in a humidified chamber. After a PBS wash, HRP-conjugated secondary antibody was applied to slides for 30 minutes. After another PBS wash, slides were incubated in DAB, washed in deionized water, counterstained with Mayer's hematoxylin and dehydrated in graded alcohols through to xylene before mounting and cover slipped.

Immunohistochemical assessment

As previously described [13], GCC expression analysis was determined by multiplying the percentage score of stained cells (0, 0-5%; 1, 6-25%; 2, 25-50%; 3, 51-75%; 4, 76-100%) by the intensity staining (0, no immunoreactivity; 1, weak; 2, moderate; 3, strong) in three to five representative areas for each section at ×200 magnification. The total score obtained (0-12) was converted to a modified scale (-, 0; +, 1-4; ++, 5-8; +++, 9-12) representing the GCC expression. For this transmembrane protein GCC, the expression of both apical membrane staining and cytoplasm staining were scored respectively.

All stained sections were scored blindly under an optical microscope (Leica) by two independent investigators that had no prior knowledge of the clinicopathological data; a consensus was achieved after joint review. However, a case was sorted as unavailable data when its section was lost or contained no intestinal tissue.

Statistical analysis

The Kruskal-Wallis test was used to compare GCC expression of all groups of specimens, followed by J. Nemenyi test for each group. Associations with GCC expression and clinico-

Table 1. Clinicopathological characteristics stratified by GCC expression in colorectal cancer patients

Characteristic	Total n=390	Membranous staining				*P*	Cytoplasmic staining				*P*
		-	+	++	+++		-	+	++	+++	
Gender*						0.552					0.024
Male	227	7	22	59	139		5	139	61	22	
Female	163	4	16	38	105		5	115	35	8	
Age (years)*						0.851					0.439
< 60	249	5	25	63	156		5	162	59	23	
≥ 60	141	6	13	34	88		5	92	37	7	
Position*						0.072					0.169
Colon cancer	163	5	13	34	111		7	97	38	21	
Rectal cancer	227	6	25	63	133		3	157	58	9	
T stage**						0.001					0.143
T1	5	0	2	1	2		1	3	1	0	
T2	42	4	6	17	15		3	30	4	5	
T3	340	7	30	78	225		6	218	91	25	
T4	3	0	0	1	2		0	3	0	0	
N stage**						0.000					0.116
N0	177	11	26	50	90		6	121	43	7	
N1	142	0	9	31	102		3	90	34	15	
N2	71	0	2	16	52		1	43	19	8	
M stage*						0.952					0.987
M0	355	11	36	85	223		9	231	89	26	
M1	35	0	2	12	21		1	23	7	4	
TNM**						0.000					0.003
I	27	4	7	12	4		4	21	1	1	
II	140	7	18	34	81		2	93	40	5	
III	188	0	11	39	138		3	117	48	20	
IV	35	0	2	12	21		1	23	7	4	
Tumor differentiation*						0.361					0.248
Well, Moderate	351	11	31	87	222		8	227	88	28	
Poor, Mucinous	39	0	7	10	22		2	27	8	2	
Serum CEA*						0.432					0.346
< 5 ng/mL	244	5	24	59	156		5	165	58	16	
≥ 5 ng/mL	146	6	14	38	88		5	89	38	14	
Serum CA199*						0.060					0.010
< 37 U/mL	323	9	31	87	196		7	203	86	27	
≥ 37 U/mL	63	1	6	9	47		3	48	9	3	
Recurrence*						0.069					0.307
No	267	10	30	67	160		6	171	67	23	
Yes	105	1	7	25	72		4	70	25	6	

*Using the Mann-Whitney *U* test; **Using the Kruskal-Wallis *H* test.

pathological variables were analyzed by non-parametric analysis. The expression of GCC and OS/DFS/recurrence time were estimated by Kaplan Meier method. The log-rank test and Cox proportional hazard models were used to assess the association of GCC expression and clinicopathological variables with time-to-event outcome. Two tailed *P* values of 0.05 or less were considered to be statistically significant. Data analysis was carried out using the Linux SAS software, version 9.3 (SAS Institute, Cary, NC, USA).

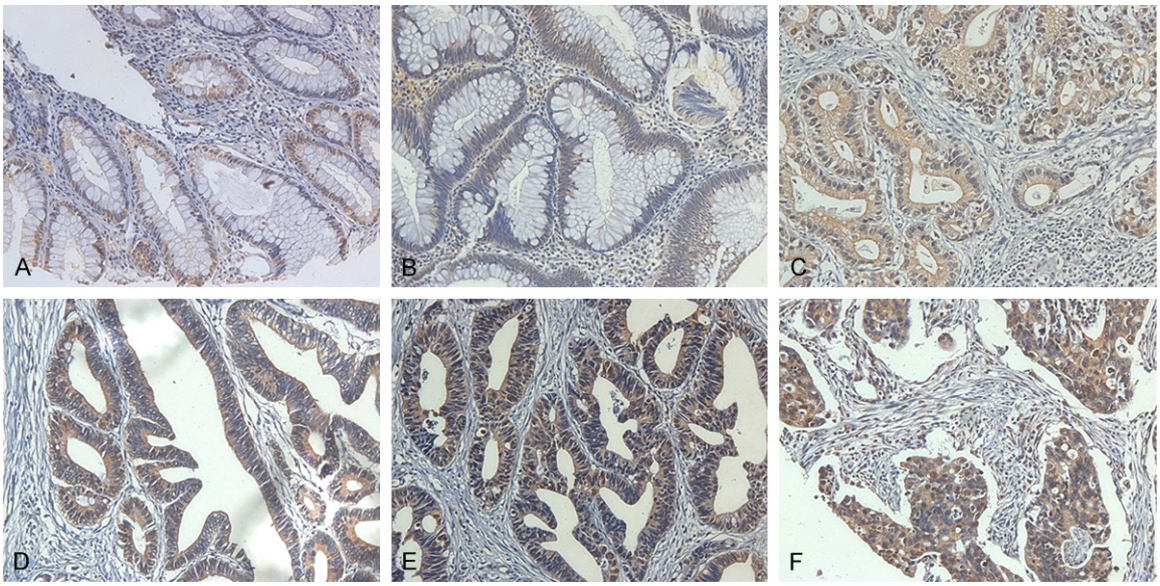

Figure 1. Representative stained cases of GCC expression in different developing stages of colorectal cancer were shown (×200). Staining for GCC showed on the apical membrane and in the cytoplasm of epithelial cells and tumor cells. Positive cells were stained brown. Both apical membranous staining and cytoplasmic staining of each case were in the same expression level. A: Low intensity expression in normal mucosa scored as "+"; B: Low intensity expression in adenoma scored as "+"; C: Moderate intensity expression in stage I tumor scored as "++". D: High intensity expression in stage II tumor scored as "+++". E: High intensity expression in stage III tumor scored as "+++"; F: High intensity expression in stage IV tumor scored as "+++".

Table 2. Expression of GCC in normal mucosa, adenoma and I~IV colorectal cancer

Group	Characteristic	Total n=476	Membranous staining				Mean rank*	P**	Cytoplasmic staining				Mean rank*	P**
			-	+	++	+++			-	+	++	+++		
1	Normal mucosa	39	4	29	6	0	80.72	-	3	24	12	0	228.81	-
2	Adenoma	47	11	22	11	3	99.30	0.993[a]	3	40	4	0	183.14	0.637[a]
3	Stage I	27	4	7	12	4	149.37	0.745[b]	4	21	1	1	169.31	0.999[b]
4	Stage II	140	7	18	34	81	255.93	0.006[c]	2	93	40	5	244.76	0.078[c]
5	Stage III	188	0	11	39	138	299.06	0.094[d]	3	177	48	20	258.15	0.954[d]
6	Stage IV	35	0	2	12	21	275.00	0.956[e]	1	23	7	4	246.41	0.997[e]

*Kruskal-Wallis H test was used to determine significances among six groups ($P < 0.001$); **Nemenyi test was used to determine significance between each two groups; [a]compared with the Normal mucosa group (Group 2 vs. Group 1); [b]compared with the Adenoma group (Group 3 vs. Group 2); [c]compared with the Stage I group (Group 4 vs. Group 3); [d]compared with the Stage II group (Group 5 vs. Group 4); [e]compared with the Stage III group (Group 6 vs. Group 5).

Results

As our related work described previously, flow chart of specimen selection of 476 cases were included. The median age of all cases was 60 (rang, 27-95) years. Of the 390 cases, 163 were located in colon and 227 in rectum. Demographic clinicopathological characteristics including gender, age, tumor position, invasive depth, lymph node metastasis, distant metastasis, TNM, tumor differentiation, serum CEA, serum CA199 and recurrence were summarized in **Table 1**.

GCC expression in normal Mucosa, Adenoma, I~IV CRC

Expression of GCC, a transmembrane protein receptor, showed both apical membranous staining and cytoplasmic staining scored as -, +, ++, +++ in cases examined. Different staining levels of GCC expressed on the apical membrane and in the cytoplasm of epithelial cells were show in **Figure 1**. The expression (membranous vs. cytoplasmic) of GCC in different developing stages of CRC was shown in **Table 2**. During the process of normal mucosa, ade-

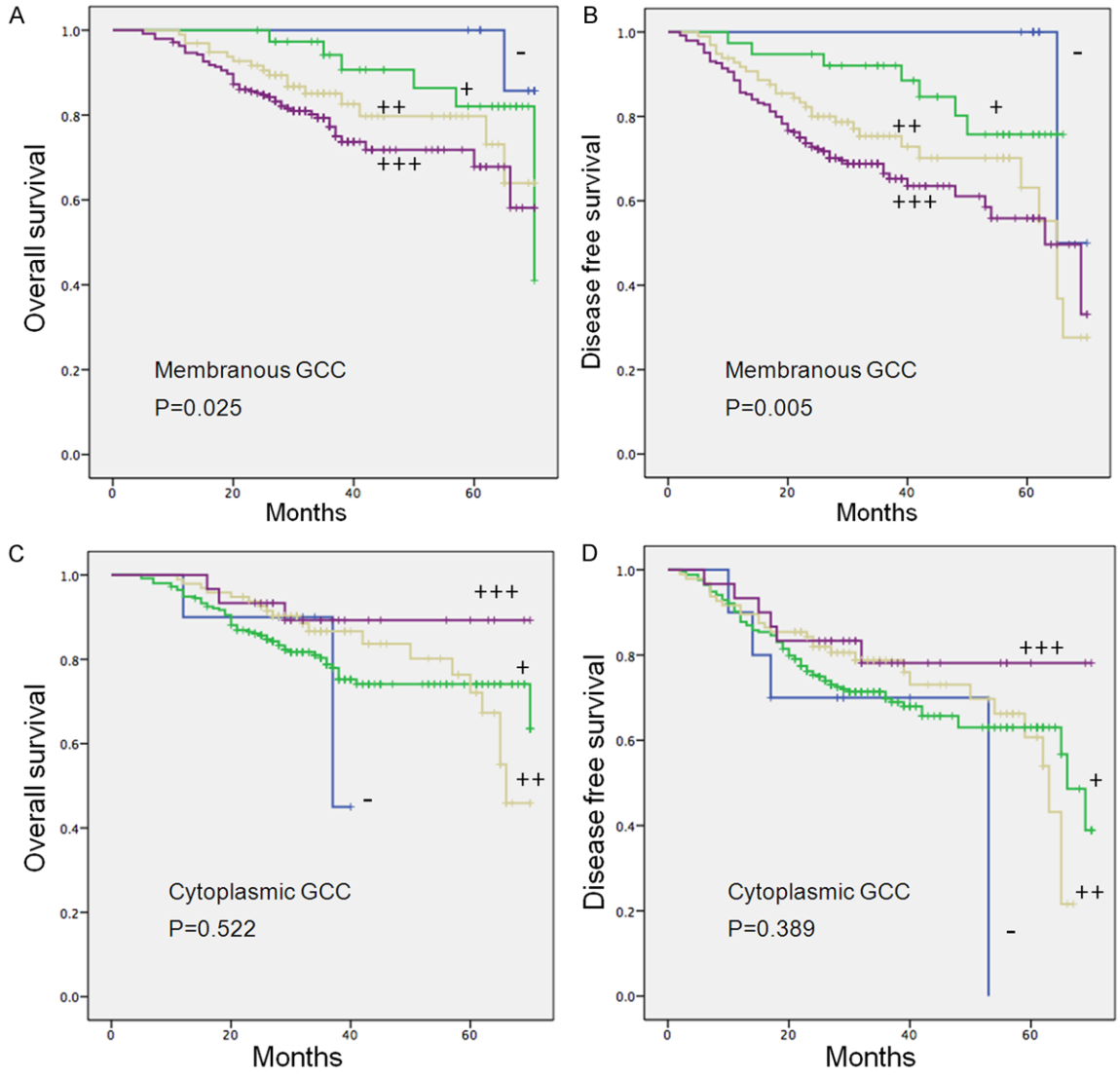

Figure 2. Kaplan-Meier survival curve in colorectal cancer patients according to the expression level of GCC. A. Overall survival of membranous GCC expression, P=0.025; B. Disease-free survival of membranous GCC expression, P=0.005; C. Overall survival of cytoplasmic GCC expression, P=0.522; D. Disease-free survival of cytoplasmic GCC expression, P=0.389. Abbreviation: GCC, guanylyl cyclase C.

noma, stage I, stage II, stage III and stage IV, the positive rate of GCC expression (membranous vs. cytoplasmic) was respectively (89.7%, 76.6%, 85.2%, 95.0%, 100.0%, 100.0%) vs. (92.3%, 93.6%, 85.2%, 98.6%, 98.8%, 97.1%). There was significant difference in both membranous and cytoplasmic expression of GCC among six groups (Kruskal-Wallis test, $P < 0.01$ vs. $P < 0.01$). Moreover, Nemenyi tests showed that membranous GCC expression level in stage II CRC was significantly higher than that in stage I CRC (P=0.006). However, there were no significances in cytoplasmic expression of GCC between each adjacent stage in the development of CRC.

Association with GCC expression and clinicopathological parameters in CRC patients

As shown in **Table 1**, GCC membranous overexpression was associated with higher TNM (P=0.000), higher T stage (P=0.001) and higher N stage (P=0.000). Spearman correlation coefficients between GCC membranous expression and TNM, T stage, N stage were respectively 0.250 (P=0.000), 0.207 (P=0.000), 0.238 (P=0.000). No significant association was observed

Table 3. Univariate association between clinicopathological parameters and outcomes (n=386)

Covariate	Overall survival			Disease free survival		
	Hazard ratio	95% CI	P	Hazard ratio	95% CI	P
Gender						
Male, female	0.945	0.602-1.485	0.806	0.815	0.563-1.182	0.281
Age						
< 60, ≥ 60)	1.456	0.932-2.275	0.099	1.036	0.714-1.503	0.853
TNM						
I, II, III, IV	2.214	1.599-3.066	0.000	1.992	1.530-2.593	0.000
Differentiation						
Well/moderate, Poor/mucinous	1.962	1.058-3,638	0.032	1.533	0.890-2.643	0.124
Position						
Colon, rectal	0.408	0.258-0.646	0.000	0.665	0.464-0.954	0.027
CEA						
< 5 ng/mL, ≥ 5 ng/mL	1.708	1.096-2.663	0.018	1.651	1.152-2.366	0.006
CA199						
< 37 U/mL, ≥ 37 U/mL	2.126	1.286-3.516	0.003	1.906	1.252-2.901	0.003
GCC membranous expression						
-, +, ++, +++	1.633	1.183-2.254	0.003	1.614	1.233-2.111	0.000
GCC cytoplasmic expression						
-, +, ++, +++	0.774	0.538-1.114	0.168	0.795	0.595-1.062	0.120
Post treatment						
No, Yes	2.381	1.094-5.181	0.029	2.867	1.499-5.481	0.001

Abbreviation: CI, confidence interval; GCC, guanylyl cyclase C.

in GCC membranous expression compared with gender, age, position, M stage, tumor differentiation, serum CEA, serum CA199 and incidence of recurrence.

Intriguingly, GCC cytoplasmic expression was significantly related to TNM (P=0.003), serum CA199 (P=0.010) and gender (P=0.024), with respective Spearman correlation coefficient were 0.118 (P=0.020), -0.084 (P=0.101), and -0.114 (P=0.024). No significant association was found in GCC cytoplasmic expression compared with age, position, T stage, N stage, M stage, tumor differentiation, serum CEA and incidence of recurrence.

Association with GCC expression and overall survival (OS) and disease-free survival (DFS) in CRC patients

As showed in **Figure 2**, Kaplan-Meier survival curves elucidated that GCC membranous expression was positively associated with OS (**Figure 2A**, P=0.025) and DFS (**Figure 2B**, P=0.005), whereas GCC cytoplasmic expression was negatively associated with OS (**Figure 2C**, P=0.522) and DFS (**Figure 2D**, P=0.389).

Univariate Cox proportional hazard analyses (**Table 3**) demonstrated that advanced TNM (P=0.000), poor differentiation (P=0.032), colonic tumor (P=0.000), elevated serum CEA (P=0.018), elevated serum CA199 (P=0.003), higher GCC membranous expression (P=0.003) and postoperative radiochemotherapy (P=0.029) were as independent predictive factors for shorter OS. Meanwhile advanced TNM (P=0.000), colonic tumor (P=0.027), elevated serum CEA (P=0.006), elevated serum CA199 (P=0.003), GCC membranous expression (P=0.000) and postoperative radiochemotherapy (P=0.001) were demonstrated (**Table 3**) as independent predictive factors for shorter DFS.

Furthermore, multivariate Cox proportional hazard analyses were observed in **Table 4**. Higher TNM (P=0.009 and P=0.020) and higher GCC membranous expression (P=0.014 and P=0.018) were demonstrated as predictive hazard factors for poor prognosis (both shorter OS and shorter DFS). Meanwhile multivariate analyses identified Poor differentiation (P=0.025) and colonic tumor (P=0.003) as predictive factors for shorter OS as well as elevated CEA

Table 4. Multivariate association between clinicopathological parameters and outcomes (n=386)

Covariate	Overall survival			Disease free survival		
	Hazard ratio	95% CI	P	Hazard ratio	95% CI	P
Gender						
Male, female	0.676	0.415-1.101	0.116	0.684	0.464-1.008	0.055
Age						
< 60, ≥ 60	1.504	0.932-2.427	0.095	1.154	0.775-1.718	0.480
TNM						
I, II, III, IV	1.755	1.152-2.675	0.009	1.516	1.069-2.152	0.020
Differentiation						
Well/Moderate, Poor/Mucinous	2.094	1.096-3.999	0.025	1.534	0.873-2.695	0.137
Position						
Colon, Rectal	0.480	0.294-0.783	0.003	0.781	0.531-1.149	0.210
CEA						
< 5 ng/mL, ≥ 5 ng/mL	1.490	0.912-2.434	0.112	1.496	1.020-2.195	0.039
CA199						
< 37 U/mL, ≥ 37 U/mL	1.335	0.765-2.330	0.309	1.294	0.819-2.045	0.269
GCC membranous expression						
-, +, ++, +++	1.549	1.092-2.198	0.014	1.410	1.060-1.876	0.018
GCC cytoplasmic expression						
-, +, ++, +++	0.636	0.438-0.922	0.017	0.693	0.516-0.929	0.014
Post treatment						
Yes, No	1.423	0.548-3.699	0.469	1.774	0.811-3.881	0.151

Abbreviation: CI, confidence interval; GCC, guanylyl cyclas.

(P=0.039) as a predictive factor for shorter DFS. Surprisingly, higher GCC cytoplasmic expression was associated with prolonged OS (HR=0.0636, 95% CI 0.438-0.922, P=0.017) and DFS (HR=0.693, 95% CI 0.516-0.929, P=0.014).

Discussion

Accurate cancer stage in diagnosis not only determines prognosis of CRC patients, but also guides selection of appropriate treatments. However, because stage II tumors are highly heterogeneous, with 5-year relative overall survivals range from 87.5% to 58.4% [14], lacking specific and sensitive techniques to evaluate lymph node micro metastases resulting in under staging to develop recurrences for approximately 30% of patients who are lymph node negative evaluated by current standard histopathology [11]. Thus, an appropriate biomarker might be great helpful to improve the accuracy of staging CRC patients.

Generally an appropriate biomarker meets two requirements. Firstly, in originating tumor or tissues, the marker has to be highly expressed. Secondly, the marker has to lack expression in tissues in which the metastatic tumor cells should be detected [15, 16]. GCC is exclusively expressed in normal intestinal mucosal cells, adenomatous polyps and colorectal cancer cells but not in extra-intestinal tissues or tumors [17]. Schulz et al. reported GCC mRNA quantified by reverse transcriptase-polymerase chain reaction (RT-PCR) was overexpressed by colorectal tumors from 41 patients, which correlated with increased GCC protein quantified by immunohistochemistry, suggesting this protein might be a sensitive and specific marker for the colorectal tumor [10]. To the best of our knowledge, the present study is the first report to explore the expression of GCC in development of colorectal cancer, using tissue microarrays (TMA), an array-based high-throughput technique that facilitates gene expression and copies number surveys of tumors.

In our study, for the trans-membranous character of GCC protein, we observed GCC expressed on both membrane and cytoplasm, elucidating an upward trend for membranous GCC expression with the stage development from normal

tissue to adenocarcinoma as well as no significantly obvious trend for cytoplasmic GCC expression. Meanwhile, the mean rank of membranous GCC expression increased consecutively in the development of CRC, especially for stage II CRC, which is significant higher than stage I CRC. These findings insinuated that membranous but not cytoplasmic GCC expression was related to tumor progression, and the high membranous GCC expression in combination with other techniques such as computed tomography (CT) might be a great importance for improving the accuracy of evaluating the undetectable micro metastasis of stage II patients. Furthermore, the high positive rate of membranous GCC expression of normal mucosa (89.7%), adenoma (79.6%), especially in adenocarcinoma (ranging from 85.2% to 100%) demonstrated a fundamental status to be selected as a prognostic molecular biomarker. Besides, the relative small sample size might result in non-statistical difference between membranous GCC expression of adenoma and stage I CRC. Nonetheless, Winn et al. reported a contrary result that poorly differentiated CRC would not express GCC, suggesting that the utility of GCC expression as a diagnostic marker for colorectal carcinoma may be questionable in poorly differentiated colorectal neoplasms [6].

To accurately diagnose tumor stage, proper evaluation of tumor invasive depth and lymph node status were two crucial factors, which were mainly quantified by preoperative magnetic resonance imaging (MRI), endorectal ultrasound (US) and CT technique. However, Akbari et al. [18]. Reported to diagnose tumor stage by different image techniques, the accuracy of MRI, endorectal US and CT were respectively (55-89%, 63-93%, 53-94%) for T stage and (60-83%, 61-80%, 56-72%) for N stage, suggesting that the utility of certain image technique as a assessable standard for colorectal tumor stage might be questionable. Especially for early stage CRC, there was an obvious limitation to assess lymph node micro metastasis that possibly resulting in disease recurrence. Meanwhile, usage of colonoscopy to prove biopsy-based pathology was very important to diagnosis of malignant tumor and prediction of prognosis, whereas it was difficult to obtain accurate information about T stage and N stage to determine appropriate treatment selection, especially for whether necessity of salvage laparotomy or not. Thus, a reliable biological factor is needed to guide tumor stage. Our present results offered additional evidence that the GCC membranous expression was significantly associated with T stage and N stage, implying that GCC membranous expression might be a predictive factor of colorectal tumor stage. Consequently, for biopsy-based tissue resected under colonoscopy, immunohistochemical detection of GCC membranous expression might be useful for prognostic prediction and treatment selection in early colorectal cancer with unclear N stage.

The result of our study also indicated that GCC membranous expression was a valuable prognostic marker for colorectal cancer patients. To the best of our knowledge, the present study is the first to explore the association between the GCC expression of mainland tumor tissue and long-term survivals for colorectal cancer patients. Clinically, the most crucial determinant of predicting survivals in colorectal patients is the presence of tumor cells in lymph nodes and evaluation of those nodes for metastatic disease. Previous studies mainly focused on the value of tissue-specific GCC expression served as a biomarker for the presence of metastatic colorectal cancer cells in normal human tissues, suggesting that GCC RT-PCR detected metastatic cancer cells in lymph nodes from 1/3 of patients with Dukes B disease, in which tumor cell could not be detected by standard histopathological analysis [12, 19-21]. Waldman, et al. also reported that expression of GCC in histological negative lymph nodes appears to be independently associated with time for recurrence and disease-free survival in patients with pN0 colorectal cancer [22], presuming that many patients with recurrence harbored occult metastases not identified at the time of primary resection and molecular staging of GCC quantified by RT-PCR could overcome such limitations. Those researches alluded us whether GCC expression of mainland tumor tissues may serve as a predictive marker for colorectal cancer patients.

Hence, to confirm this assumption, Kaplan-Meier analysis was used to verify that elevated level of GCC membranous but not cytoplasmic expression was significantly related to prolonged overall survivals and disease-free sur-

vivals. Furthermore, we employed univariate and multivariate analyses to clarify its contribution to OS and DFS. The result of multivariate Cox proportional hazard analysis confirmed that GCC membranous expression was an independent hazard factor of both OS (HR=1.549, 95% CI 1.092-2.198, P=0.014) and DFS (HR= 1.410, 95% CI 1.060-1.876, P=0.018), suggesting that the elevated level of GCC membranous expression was negatively associated with improved prognosis. Surprisingly, through multivariate Cox proportional analysis, our study also indicated that GCC cytoplasmic expression might be an independently protective factor for both OS and DFS. This would also be of significance if future modalities use GCC as a target for cells imaging or therapeutic purpose for early stage colorectal cancer patients. Furthermore, this offered us a new hypothesis for our next study that the mechanism of GCC expression might be associated with different distribution of between membrane and cytoplasm in different colorectal cancer stage. However, our study has several important limitations. Firstly, possible biases may be induced by the high incidence of lost to follow-up (30.9%). Secondly, overall-survivals and disease-free-survivals may be affected by excluding the patients with neoadjuvant radiotherapy or chemotherapy to minimize the interference of chemoradiotherapy on GCC protein expression.

Conclusion

Our findings demonstrate that GCC membranous expression is overexpressed in stage II colorectal tumor. And GCC membranous expression is predictive of tumor stage, especially for predicting T stage and N stage for early colorectal cancer resected under endoscopy whose tumor stage-related factors are unavailable. Confirming that GCC membranous expression is independent prognostic factors of OS and DFS of colorectal cancer patients, that is valuable in prognosis prediction and treatment selection. These data suggest that membranous GCC may be a valuable immunohistochemical prognostic biomarker for colorectal cancer. Further study of the mechanism of GCC in human colorectal cancer is warranted.

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Disclosure of conflict of interest

None.

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