

Assessment of GCC Expression as a Prognostic Marker of Time to Recurrence in Colon Cancer

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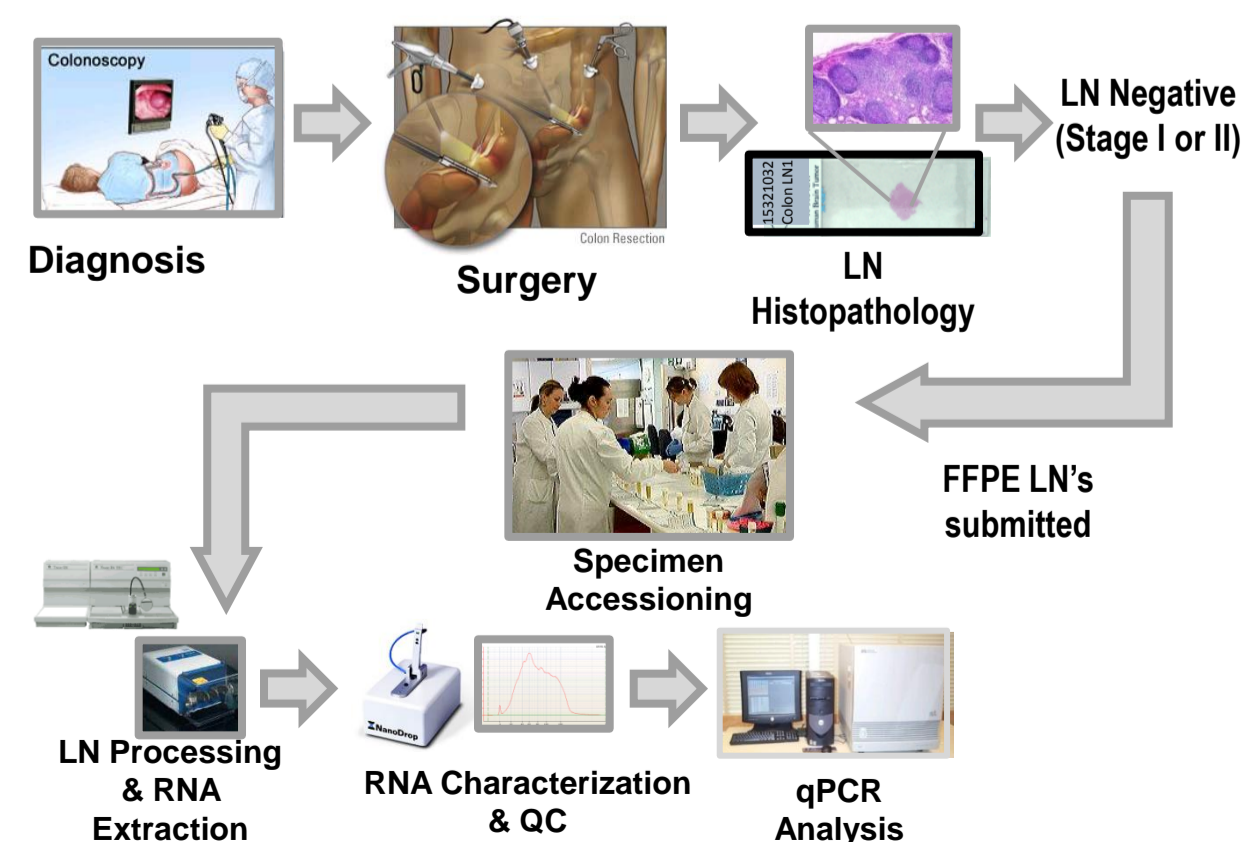
Background

Predicting outcome for colon cancer patients considered free of lymph node (LN) metastases by histopathology (HP) remains challenging. Approximately 20% of patients diagnosed with node-negative (pN0) colon cancer will relapse¹. For this reason, detection of LN metastases in patients diagnosed with colon cancer is a critical risk factor to predict disease recurrence². Dr. Scott Waldman and colleagues have shown that the Guanylyl cyclase C (GCC or GUCY2C), a receptor for bacterial diarrheagenic enterotoxins, is expressed selectively by intestinal epithelium and is an endogenous downstream target of CDX2³. The expression of GCC is preserved throughout the adenoma/carcinoma sequence in the colon and can facilitate the detection of colon cancer occult metastases in LNs classified as negative by routine HP examination⁴. The aim of this study is to decipher the relationship between GCC mRNA expression in formalin-fixed, paraffin embedded LNs (FFPE) and recurrence risk in patients with pN0 colon cancer.

Methods & Design

Association of GCC mRNA expression with outcome was evaluated in 123 cases from two independent retrospective cohorts⁵ of patients diagnosed with pN0 colon cancer without adjuvant chemotherapy.

RNA was extracted from 1724 FFPE LNs and GCC status determined using an RT-qPCR method⁶.



Results

At least one LN with a positive GCC mRNA level was found by RT-qPCR in 24 (19.5%) of the 123 patients with node-negative disease. (Figure 1)

Expression of GCC mRNA in regional LNs is related to tumor grade but not to other clinicopathologic factors such as lymphovascular invasion. (Table 1)

Overall recurrence rate for patients with pN0 disease who had at least one GCC-positive LN was 33% (8/24) compared to 16% (16/99) for GCC-negative patients (Log-Rank p=0.0278). (Figure 2)

Multivariate analysis, adjusted for age and tumor grade, shows that GCC expression is a strong independent marker for time to recurrence. (HR 3.54; 95%CI: 1.40-8.98; p=0.0077) (Table 3)

Patient Characteristics

Hôtel-Dieu de Québec (HDQ)

25 Stage I/II colon cancer patients (1999-2005)

Stage I (T1/T2): 6
Stage IIA (T3): 17
Stage IIB (T4): 2

University of Massachusetts (UMass)

98 colon cancer (1991-1998).

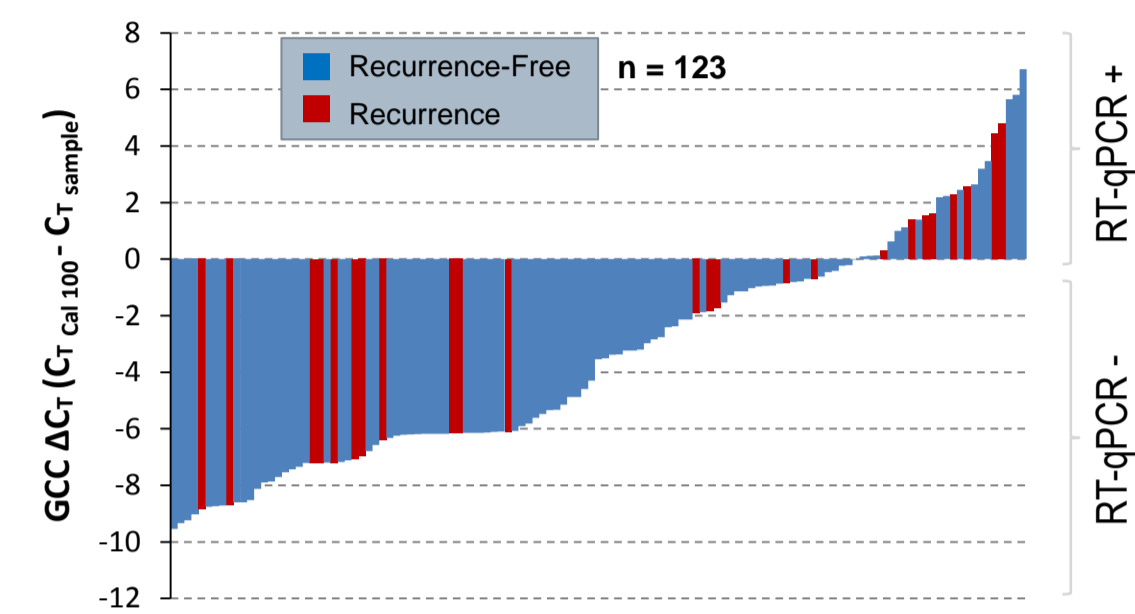
Stage IIA (T3) colon cancer patients

	Relapse (n=24)	Non-relapse (n=99)
Follow-up (months)*		
Average	28,1	57,1
Median	21,9	52,6
Range	<1 - 65	12 - 117
Age (years)		
Average	65,5	70,9
Median	67	71
Range	24 - 87	46 - 95
Nb of examined LN		
Average	13,2	14,2
Median	12,5	11
Range	2 - 30	1 - 75
GCC Status		
Negative	16 / 67%	83 / 84%
Positive	8 / 33%	16 / 16%

* For the relapsing group, the follow-up time is the interval from surgery to recurrence,

Figure 1

GCC mRNA detection rate in 123 patients with pN0 colon cancer using GCC RT-qPCR test.



A **Positive** test result (≥ 0) indicates the LN contains GCC mRNA at a level consistent with occult colorectal cancer metastases.

A **Negative** test result (< 0) indicates GCC mRNA is below the limit of detection.

33% (8/24) of patients with disease recurrence were detected using GCC mRNA RT-qPCR test.

On the other hand, 16% (16/99) of GCC-negative patients relapsed (NPV 84%; 95% CI: 77%-90%).

86% of non-relapsing patients had at least 36-months of follow-up.

Table 1

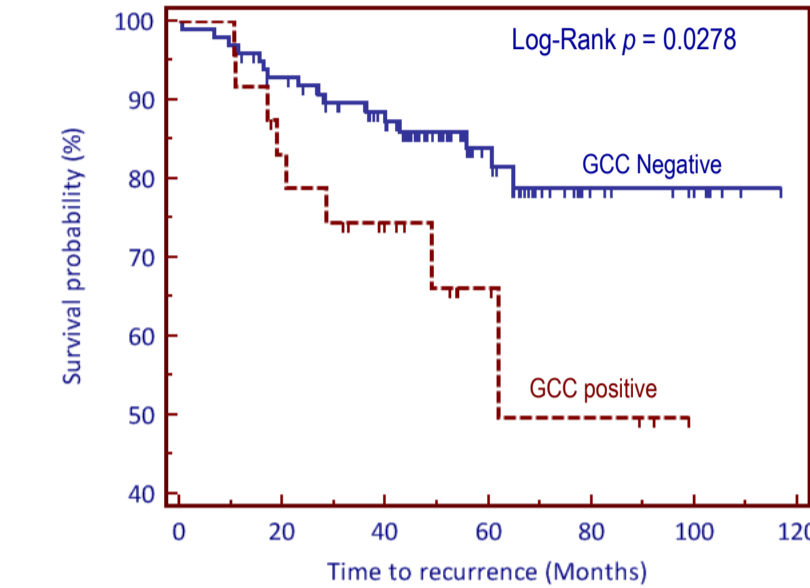
Clinicopathologic parameters of patients with node-negative colon cancer according to GCC expression.

Clinical feature	Nb of Patients (n=123)	%	Nb (%) of Patients		P Value
			GCC negative (n=99)	GCC positive (n=24)	
Age					
< 70	58	47%	47 (47%)	11 (46%)	0.8851
≥ 70	65	53%	52 (53%)	13 (54%)	
Sex					0.1591
Male	51	41%	38 (38%)	13 (54%)	
Female	72	59%	61 (62%)	11 (46%)	
Tumor Grade					0.014
Well differentiated (1)	27	22%	19 (19%)	8 (33%)	
Moderately differentiated (2)	72	59%	57 (58%)	15 (63%)	
Poorly or undifferentiated (3)	18	15%	18 (18%)	0 (0%)	
Unknown	6	5%	5 (5%)	1 (4%)	
Tumor Stage					0.0879
Stage I (T1/T2)	6	5%	3 (3%)	3 (13%)	
Stage II (T3/T4)	117	95%	96 (97%)	21 (88%)	
LVI					0.6819
No	81	66%	64 (65%)	17 (71%)	
Yes	34	28%	29 (29%)	5 (21%)	
Unknown	8	7%	6 (6%)	2 (8%)	
Tumor Location *					0.0754
Proximal	82	67%	70 (71%)	12 (52%)	
Distal	39	32%	28 (29%)	11 (48%)	
Nb of examined LN					0.3865
< 10	42	34%	32 (32%)	10 (42%)	
≥ 10	81	66%	67 (68%)	14 (58%)	
Years of diagnosis					0.2944
1991 - 1997	78	63%	65 (66%)	13 (54%)	
1998 - 2005	45	37%	34 (34%)	11 (46%)	

* Proximal colon includes cecum to transverse colon and distal colon includes splenic flexure to sigmoid colon. Tumor location was unknown in only two (2) cases.

Figure 2

Recurrence-free survival in 123 patients with pN0 colon cancer employing GCC RT-qPCR result as a categorical (positive/negative) variable.



Recurrence rate:

36 months:
GCC Negative: 10% (10/99)
GCC Positive: 25% (6/24)

Overall:
GCC Negative: 16% (16/99)
GCC Positive: 33% (8/24)

Number at risk	0	20	40	60	80	100	120
GCC Neg: 99	89	71	35	13	6	1	
GCC Pos: 24	19	12	5	3	0	0	

Table 2

Univariate Cox proportional hazards regression of time to recurrence in patients with pN0 colon cancer.

Clinical and molecular features	Nb of Patients (n=123)	Nb of Events (n=24)	RFS (%)	Univariate HR (95% CI)	P Value
Age					
< 70	58	14	76%	1,00 (Reference)	
≥ 70	65	10	85%	0,97 (0,92 - 1,01)	0,1518
Sex					
Male	51	13	75%	1,00 (Reference)	
Female	72	11	85%	0,57 (0,26 - 1,28)	0,1742
Tumor Grade					
Well and Moderate (1 and 2)	99	18	82%	1,00 (Reference)	
Poor and Undiff. (3 and 4)	18	6	67%	2,07 (0,82 - 5,22)	0,1244
Tumor Stage					
Stage I (T1/T2)	6	2	67%	1,00 (Reference)	
Stage II (T3/T4)	117	22	81%	0,60 (0,14 - 2,55)	0,4868
LVI					
No	81	14	83%	1,00 (Reference)	
Yes	34	9	74%	1,56 (0,68 - 3,62)	0,2954
Tumor Location *					
Proximal	82	12	85%	1,00 (Reference)	
Distal	39	10	74%	1,80 (0,78 - 4,17)	0,1706
Nb of examined LN					
< 10	42	7	83%	1,00 (Reference)	
≥ 10	81	17	79%	1,27 (0,53 - 3,06)	0,5979
Years of diagnosis					
1991 - 1997	78	15	81%	1,00 (Reference)	
1998 - 2005	45	9	80%	1,17 (0,51 - 2,70)	0,7070
Presence of GCC (CO: $\Delta Ct = 0$)					
No	99	16	84%	1,00 (Reference)	
Yes	24	8	67%	2,53 (1,07 - 5,94)	0,0337

* Proximal colon includes cecum to transverse colon and distal colon includes splenic flexure to sigmoid colon. Tumor location was unknown in only two (2) cases.

Table 3

Multivariate Cox proportional hazards model analyses of time to recurrence for presence of GCC together with clinicopathologic features.

Clinical and molecular features	Nb of Patients (n=123)	Nb of Events (n=24)	All Patients Multivariate HR (95% CI)	P Value
Age				
< 70	58	14	1,00 (Reference)	
≥ 70	65	10	0,62 (0,28 - 1,41)	0,2567
Tumor Grade				
Well and Moderate (1 and 2)	99	18	1,00 (Reference)	
Poor and Undiff. (3 and 4)	18	6	3,48 (1,36 - 8,93)	0,0094
Presence of GCC				
No	99	16	1,00 (Reference)	
Yes	24	8	3,54 (1,40 - 8,98)	0,0077

Conclusions

When tested for the presence of GCC mRNA, 19.5% (24/123) of patients with pN0 colon cancer were GCC-positive. (Figure 1)

Expression of GCC mRNA in LNs was observed more frequently in well and moderately differentiated tumors than in poor and undifferentiated ones (p=0.014). (Table 1)

GCC-positive patients were 2 times more likely to relapse than GCC-negative patients. (Figure 2)

There were less patients with recurrence-free survival among those with RT-qPCR positive nodes (67% vs 84%; p=0.0337) (Table 2)

With that cohort of HP node-negative colon cancer patients, detection of GCC mRNA in regional LNs was associated with an higher risk of earlier recurrence.

GCC mRNA test could identify the presence of occult nodal metastases, and predict the likelihood of disease recurrence in patients diagnosed with pN0 colon cancer.

The potential of GCC as a prognostic biomarker for patients with Stage I-II disease is currently under validation in a large clinical study with higher statistical power.

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