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Low expression of CysLT$_1$R and high expression of CysLT$_2$R mediate good prognosis in colorectal cancer

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ABSTRACT

Colorectal cancer is the third most common cancer type in the Western world. In search of new treatment possibilities, the inflammation mediators, know as cysteinyl leukotrienes (CysLTs), have been shown to regulate intestinal epithelial cell survival and proliferation via the CysLT$_1$R, and cell differentiation via the CysLT$_2$R. These results prompted us to investigate the significance of CysLT$_1$R and CysLT$_2$R expression in colorectal cancer tissue for patient survival. The CysLT$_1$R, CysLT$_2$R, β-catenin and Bcl-xL protein expression levels were evaluated by immunohistochemistry in a tissue microarray of 329 colorectal patients. We found that high nuclear expression of CysLT$_1$R is associated with a poor prognosis, whereas high nuclear expression of CysLT$_2$R is associated with a good prognosis. We also observed that patients with colorectal tumours characterised by high CysLT$_1$R but low CysLT$_2$R nuclear expression had the lowest survival expectancy, whereas patients with colorectal tumours characterised by low CysLT$_1$R but high CysLT$_2$R nuclear expression had the best survival expectancy. Interestingly, β-catenin as a single prognostic marker did not exhibit any prognostic value. However, in patients with tumours characterised by a high CysLT$_1$R nuclear expression, an elevated β-catenin nuclear expression had a significantly prognostic value.

In conclusion these data indicate that nuclear expressions of CysLTRs are potential prognostic indicators of colorectal cancer.

1. Introduction

Colorectal cancer is the third most common cancer in the Western world. Since almost half of colorectal cancer patients die of metastatic disease, studies about the molecular mechanisms and an evaluation of their prognostic role are needed. Virchow speculated about the role of chronic inflammation in cancer nearly 150 years ago based on his observation that hematopoietic cells frequently infiltrated neoplastic tissue.[1] More and more evidence have shown that a multifactor microenvironment of immune cells and signalling molecules interact with, and influence, neoplastic cells in all stages of tumourigenesis. Moreover, chronic inflammation in the gut is an established risk factor for colorectal cancer

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development, which increases the risk of developing colon cancer by 30%.[2]

Inflammatory bowel disease (IBD) and cancer development are linked through the activation of arachidonic acid (AA) metabolites and enzymes responsible for the metabolic pathway of AA.[3] Cyclooxygenases (COX-1, -2) convert AA into prostaglandins (PGs), and COX-2 along with PGs increase with both inflammation and cancer.[4] The same was reported for 5-lipoxygenase (5-LO), which converts AA into leukotrienes (LTs) and for LTs themselves.[5,6] Furthermore, patients treated with COX-2 inhibitors show reduced progression of colon cancer.[7,8] We have previously shown that the levels of COX-2 are upregulated in colon cancer tissues, cell lines, and in a non-transformed intestinal cell line after stimulation with the pro-inflammatory mediator leukotriene D_4 (LTD_4).[9] LTD_4 belongs to the group of cysteinyl leukotrienes (CysLTs), together with LTC_4 and LTE_4. They act primarily via specific seven transmembrane receptors on the target cells. Three such receptors have been cloned; $CysLT_1$,[10] $CysLT_2$[11] and, more recently, the orphan receptor GPR17.[12] Of these three receptors, $CysLT_1R$ has the highest affinity for LTD_4. In inflammation, leukotrienes affect smooth muscle contraction, mucus production and chemotaxis.[13] Recently, we observed that LTD_4 via $CysLT_1R$ increases cell survival signalling in intestinal epithelial cells, and that low levels of leukotriene signalling are necessary for cell homoeostasis.[14,15] We have also shown that LTD_4 could increase the levels of the anti-apoptotic protein Bcl-2 and β-catenin, which can influence the apoptosis rate.[16,17] Additionally, signalling via $CysLT_1R$ also mediates increased cell proliferation[18] and increased cell migration.[19] Conversely, signalling via $CysLT_2R$ does not appear to drive the tumourigenic process. Instead, LTC_4, acting via $CysLT_2R$, is involved in cell differentiation.[20]

Moreover, we have shown that in intestinal epithelial cells, LTD_4 stimulates the translocation of free β-catenin, both to the nucleus, where it activates the TCF/LEF promoter, and to the mitochondria, where it affects survival.[21] β-catenin is an approximately 94-kDa protein that has many distinct functions. When present at the plasma membrane, β-catenin forms adherence-type junctions by linking cadherins to the actin cytoskeleton.[21] Furthermore, β-catenin is an effector molecule of the Wnt-signalling pathway, which is important in both embryonic development and carcinogenesis.[22,23] When Wnt signalling is absent, β-catenin expression is tightly regulated by the protein complex, adenomatous polyposis coli (APC)/glycogen synthase kinase-3β (GSK-3β) and axin. Loss of this regulation results in nuclear translocation of β-catenin, where it induces the transcription of genes involved in proliferation.[22]

In this study, we have examined CysLTRs and β-catenin expression in colorectal cancer and the impact of increased protein expression on the survival of colorectal cancer patients.

2. Materials and methods

2.1. Patients and specimens

Tumour biopsies obtained before any treatment were used in this study. The study included 329 colorectal patients in total. The material was collected from consecutively patients admitted to Malmö Univeristy Hospital in 1990 or from patients admitted to Lund University Hospital between 1994 and 2000. All patients were treated by surgical removal of their tumour. However, tumour stage then dictated the treatment, but combination chemotherapy was standard, and none of the included patients received antibody treatment. Specimens of 79 colon tumours and 245 rectal tumours were grouped into proximal (proximal colon) and distal (distal colon and rectum). The colorectal tumour specimens and matching healthy epithelium were obtained from the archives of the Department of Pathology at Malmö University Hospital, and the Department of Oncology at Lund University Hospital. The rectal tumour specimens have previously been described in [24] and the colon tumour specimens have been described in [25]. The differentiation grade was low in 99 colorectal cancer patients, moderate in 242 patients and high in eight patients. Staging was performed according to Dukes' classification,[26] and according to four different TNM-based stages suggested by the World Health Organisation.[27,28] Histopathologic characteristics of the specimens are summarised in Tables 1, 2 and 4. Patients who died of causes other than colorectal cancer were censored at the time of death, shown in Figs. 2 and 3. Overall survival was used as the clinical endpoint, and the minimum follow-up time for survivors was 120 months. Permission for the study was granted by the Lund University Ethics Committee.

2.2. Tissue arraying and immunohistochemistry

Sections from tissue microarray (TMA) blocks were deparaffinised, rehydrated and stained with haematoxylin-eosin. Each tumour was represented in triplicate in the array, and immunohistochemical procedures were performed using a Dako automatic slide stainer according to manufacturers' instructions. This was followed by counterstaining with haematoxylin.

2.3. Antibodies

The tissue array was stained with rabbit polyclonal anti-human $CysLT_1R$ (dilution 1:600) and $CysLT_2R$ (dilution 1:100–1:300) antibodies from Innovagen, Lund, Sweden, and with anti-β-catenin (diluted 1:1000) and Bcl-xL (dilution 1:50–1:100) from Transduction Laboratories, Lexington, KY, USA.

2.4. Scoring

Two investigators (EL and MM) evaluated all slides, and any disagreement between these observers (<10%) was reviewed until a final decision was reached. In summary, $CysLT_1R$, $CysLT_2R$ and β-catenin were assessed using an arbitrary scale, comprising four different levels of staining intensity and amount of positive cells, designated as follows: absent (–), weak (+), moderate (++) and high (+++). The latter was further grouped into two categories: low (–/+) and high (++/+++). For Bcl-xL, a 2-graded scale was used: negative or less staining than seen in a normal (control) colon (–) and staining higher than the control (+). Furthermore, the duration of follow-up

Table 1 – Distribution of nuclear CysLT$_1$R staining in colorectal tumours according to clinico-pathologic and molecular parameters.

Relative staining	CysLT$_1$R nuclear staining		*n*	*p*-Value
	Low	High		
n (%)	190 (77.6)	55 (22.4)	245 (100)	
Sex				0.059[b], 0.043[c]
Male	122	27	149 (60.8)	
Female	68	28	96 (39.2)	
Age				
Median	68.7	72.2		<0.001[d]
Range	33–90	37–85		
⩽59	28	6	34 (13.9)	0.001[a]
60–69	56	6	62 (25.3)	
70–79	89	29	118 (48.2)	
⩾80	15	13	28 (11.4)	
Missing cases: 3			3 (1.2)	
Tumour localisation				<0.001
Proximal colon	12	31	43 (17.6)	
Distal colon	5	20	25 (10.2)	
Rectum	173	3	176 (71.8)	
Missing cases: 1			1 (0.4)	
Dukes' stages				0.014[a]
A	47	4	51 (20.8)	
B	73	23	96 (39.2)	
C & D	70	28	98 (40.0)	
Differentiation grade				0.348[a]
Low	45	16	61 (24.9)	
Medium	137	36	173 (70.6)	
High	7	3	10 (4.1)	
Missing cases:1			1 (0.4)	
CysLT$_1$R cytosol				<0.001[b,c]
Low	127	8	135 (55.1)	
High	37	46	83 (33.9)	
Missing cases: 27			27 (11.0)	
CysLT$_2$R nucleus				<0.001 [b,c]
Low	30	39	69 (28.2)	
High	109	15	124 (50.6)	
Missing cases: 52			52 (21.2)	
β-Catenin nucleus				0.016[b,c]
Low	140	24	164 66.9)	
High	47	19	66 (26.9	
Missing cases: 15			15 (6.2)	
β-Catenin cytosol				<0.001 [b,c]
Low	142	13	155 (63.3)	
High	39	30	69 (28.2)	
Missing cases: 21			21 (8.5)	
Bcl-xL				<0.001 [b,c]
Low	19	34	53 (21.6)	
High	136	21	157 (64.1)	
Missing cases: 35			35 (14.3)	

a Pearson's χ^2 test (two-sided).
b Fisher's exact test (two-sided).
c Phi coefficient.
d Mann–Whitney *U*-test (two-sided).

of groups of patients defined by the grade of inflammatory receptors (CysLT$_1$R and CysLT$_2$R) was similar. All staining evaluations refer to the tumour cells and not adjacent stroma or infiltrating inflammatory cells.

2.5. *Statistical analysis*

For statistical analysis of non-parametric data, Fischer's exact test, Phi coefficient test, Pearson's χ^2 test or Mann–Whitney *U*-

test was used. Survival curves were generated using the Kaplan–Meier method, and compared using a log-rank test. Univariate and multivariate prognostic analyses were performed with Cox proportional hazard regression model to determine the risk of death. Survival time was measured from the date of surgery to the date of death or the latest follow-up. Survival time was corrected for specific cancer-induced death. Deaths due to causes other than colorectal cancer were censored at the time of death, whereas healthy surviving patients were censored at the time of clinical discharge. A p-value of $\leqslant 0.05$ was considered statistically significant. Statistical analyses were performed using SPSS software (version 16.0; SPSS Inc., Chicago, IL, USA).

3. Results

3.1. *Immunoreactivities of the cysteinyl leukotriene receptors and survival markers in colorectal carcinomas*

The expression patterns of the CysLT receptors and β-catenin were analysed in samples from a cohort with 329 colorectal cancer patients. In this cohort, 245 tumour specimens could be analysed for nuclear $CysLT_1R$ expression, 242 for nuclear $CysLT_2R$ expression and 312 for nuclear β-catenin expression. Representative expression patterns for high versus low expression of CysLT receptors and β-catenin (Fig. 1A) are summarised in relation to nuclear $CysLT_1R$ staining in Table 1, and nuclear $CysLT_2R$ staining in Table 2. Both cysteinyl receptors have previously been reported to be expressed both at the plasma membrane and at the nuclear membrane.[20,29] In this study, 22% of patients expressed high levels of $CysLT_1R$ at the nucleus, whereas 68% had a high nuclear expression of $CysLT_2R$ (Fig. 1B). In general, $CysLT_2R$ mainly stained the nucleus and had a low cytosolic staining, whereas nuclear staining of $CysLT_1R$ was generally accompanied by high cytosolic staining (Fig. 1).

Tables 1 and 2 show clinico-pathological parameters according to nuclear $CysLT_1R$ and nuclear $CysLT_2R$, respectively. Nuclear expression of both $CysLT_1R$ and $CysLT_2R$ significantly correlated with cytosolic $CysLT_1R$, cytosolic β-catenin and Bcl-xL expressions. This is interesting, considering that earlier we have shown that LTD_4 via $CysLT_1R$ upregulates these proteins, which might influence the apoptosis rate. Bcl-2 and its closest homologue, Bcl-xl, have similar downstream effects, and can therefore replace each other. In several types of human cancer, Bcl-2 is often replaced by Bcl-xl. In addition, there was a significant correlation between nuclear $CysLT_1R$ and nuclear β-catenin expressions. High expression of $CysLT_1R$ was more often observed in the proximal colon, whereas high expression of $CysLT_2R$ was more often found in the rectum. High expression of nuclear $CysLT_1R$ was more frequent in Dukes' stages C and D, whereas there was a higher percentage of patients with high nuclear expression of $CysLT_2R$ in Dukes' stage A, compared to Dukes' stages B, C and D. Due to the different expression patterns of nuclear $CysLT_1R$ and $CysLT_2R$, there was a significant inverse correlation between the expression of these two nuclear receptors.

3.2. *Cysteinyl leukotriene receptors' expression and patient survival*

High $CysLT_1R$ expression patterns have previously been implicated in poorer prognoses for colorectal cancer patients.[25] This study, with a larger number of participants, corroborates

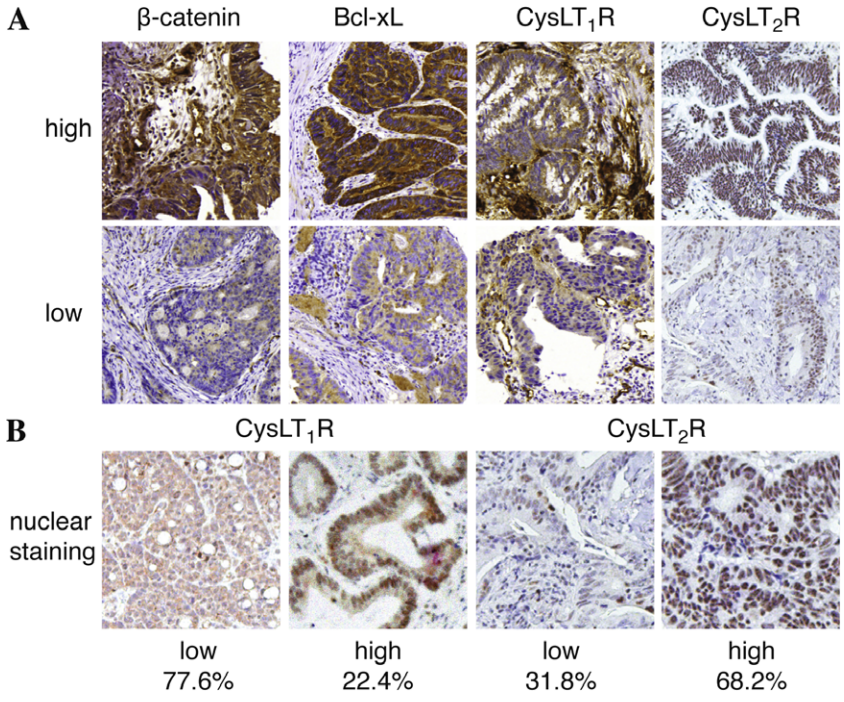

Fig. 1 – Immunohistochemical staining of β-catenin, Bcl-xL, $CysLT_1R$ and $CysLT_2R$ in colorectal tumour specimens. (A) Representative over all high and low staining. (B) Representative low, respectively, high nuclear staining of $CysLT_1R$ and $CysLT_2R$.

Table 2 – Distribution of nuclear CysLT$_2$R staining in colorectal tumours according to clinico-pathologic and molecular parameters.

Relative staining	CysLT$_2$R nuclear staining		*n*	*p*-Value
	Low	High		
n (%)	77 (31.8)	165 (68.2)	242 (100)	
Gender				0.669[b]
Male	47	106	153 (63.2)	
Female	30	59	89 (36.8)	
Age				
Median	71.6	69.1		0.095[c]
Range	37–89	33–88		
⩽59	5	27	32 (13.2)	0.192[a]
60–69	20	41	61 (25.2)	
70–79	39	74	113 (46.7)	
⩾80	12	20	32 (13.2)	
Missing cases: 4			4 (1.7)	
Tumour localisation				<0.001[a]
Proximal colon	33	12	45 (18.6)	
Distal colon	22	4	26 (10.7)	
Rectum	21	149	170 (70.3)	
Missing cases: 1			1 (0.4)	
Dukes' stages				0.037[a]
A	10	42	52 (21.5)	
B	36	54	90 (37.2)	
C & D	31	69	100 (41.3)	
Differentiation grade				0.591[a]
Low	20	41	61 (25.2)	
Medium	56	116	172 (71.1)	
High	1	8	9 (3.7)	
CysLT$_1$R cytosol				<0.001[c]
Low	21	116	137 (56.6)	
High	46	40	86 (35.5)	
Missing cases: 19			19 (7.9)	
β-Catenin nucleus				0.188[c]
Low	42	122	164 (76.8)	
High	22	41	63 (26.0)	
Missing cases: 15			15 (6.2)	
β-Catenin cytosol				<0.001[c]
Low	29	120	149 (61.6)	
High	34	42	76 (31.4)	
Missing cases: 17			17 (7.0)	
Bcl-xL				<0.001[c]
Low	41	19	60 (24.8)	
High	35	134	169 (69.8)	
Missing cases: 13			13 (5.4)	

a Pearson's χ^2 test (two-sided).
b Fisher's exact test (two-sided) (the Phi coefficient gave equivalent *p*-values).
c Mann–Whitney *U*-test (two-sided).

our previous assumptions. Patients with high cytosolic expression of CysLT$_1$R show a trend of poorer prognosis (Fig. 2A), and patients with high nuclear CysLT$_1$R expression have significantly shorter survival expectancy (Fig. 2B). This trend can be clearly seen in Fig. 2C, where patients with no detectable nuclear CysLT$_1$R expression exhibit the best survival prognosis, and patients with high nuclear CysLT$_1$R expression exhibit the poorest prognosis. The opposite trend was observed for nuclear CysLT$_2$R expression. Patients who exhibited high nuclear expression of CysLT$_2$R in their tumours had significantly better survival expectancy than patients with low nuclear CysLT$_2$R expression (Fig. 2D). Interestingly, it appears that low nuclear expression of CysLT$_1$R combined with high nuclear expression of CysLT$_2$R is a predictor of more favourable prognoses for colorectal cancer patients (Fig. 2E).

Univariate prognostic analyses according to the Cox proportional hazard regression model confirm these results. High

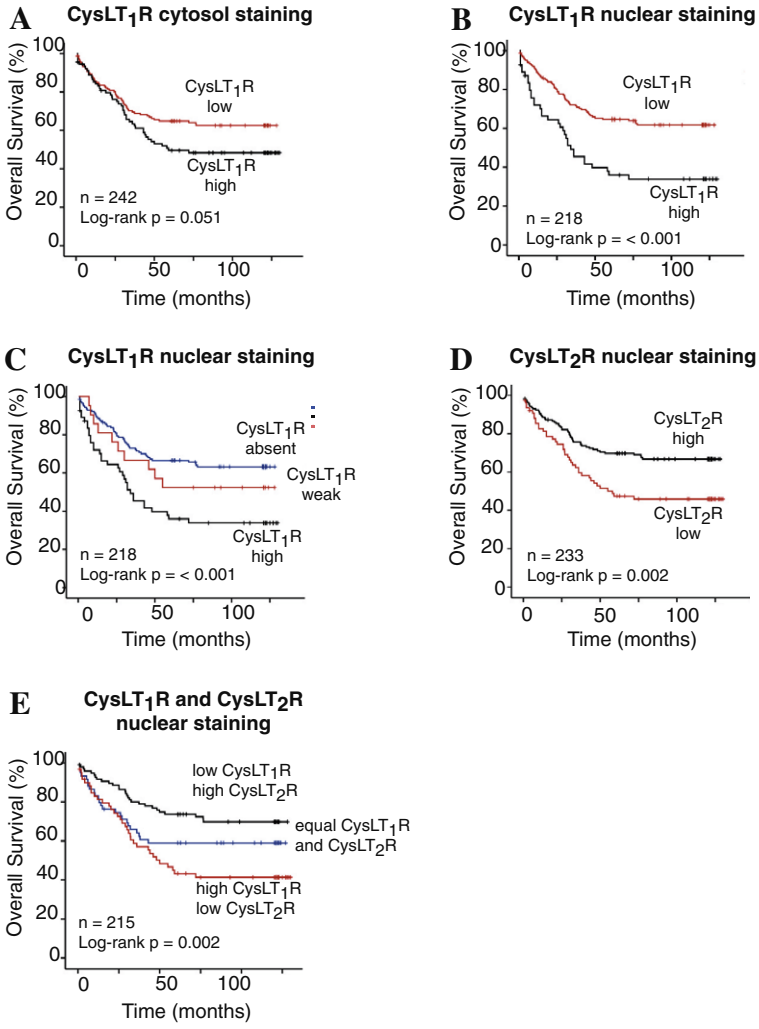

Fig. 2 – Low levels of $CysLT_1R$ and high levels of $CysLT_2R$ correlate with longer survival time. (A) Kaplan–Meier survival curve considering cytosolic $CysLT_1R$, divided into low (absent and weak) and high (medium and strong) expression. (B) Nuclear $CysLT_1R$ staining divided into low (absent and weak) and high (medium and strong) expression. (C) Nuclear $CysLT_1R$ staining divided into absent, weak and high expression. (D) Nuclear $CysLT_2R$ staining, divided into low and high expression. (E) Patient expressing nuclear $CysLT_1R$ and $CysLT_2R$, divided into three subgroups: low $CysLT_1R$ and high $CysLT_2R$, equal expression of $CysLT_1R$ and $CysLT_2R$ and high $CysLT_1R$ and low $CysLT_2R$ expression. Patient survival is shown in months and percentage survival for each group was compared using the log-rank test (pooled over strata), missing values (A) $n = 87$; (B) $n = 111$; (C) $n = 111$; (D) $n = 96$; and (E) $n = 114$.

expression levels of $CysLT_1R$ are associated with a higher risk of death (hazard ratio (HR) 2.26; 95% confidence interval (CI) 1.49–3.43). High expression of $CysLT_2R$ on the other hand is associated with a decreased risk of death (HR 0.552; 95% CI 0.364–0.836). Tumour stage and tumour location in the bowel are also significant risk factors in colorectal cancer (Table 3). In addition, we performed multivariate analyses to distinguish which of these variables were independent prognostic risk factors in colorectal cancer. Nuclear $CysLT_1R$ was an independent prognostic factor (HR 1.93; 95% CI 1.06–3.49), whereas $CysLT_2R$ lost its significant prognostic value (Table 3). Patients over 69 years frequently expressed more nuclear $CysLT_1R$. However, nuclear $CysLT_1R$ was not dependent on age as a prognostic marker. These results indicate that $CysLT_1R$ can be used as an independent prognostic marker in colorectal cancer, whereas $CysLT_2R$ is a dependent prognostic marker. It appears as though the prognostic value of $CysLT_2R$ is dependent on nuclear $CysLT_1R$ expression.

3.3. *β-Catenin survival and expression associations*

There was a significant correlation between the β-catenin expression pattern in the nucleus and in the cytosol ($p < 0.001$) in the colorectal cancer patient cohort (Table 4). β-Catenin as a single prognostic marker did not exhibit any prognostic value when examined for low versus high expression in either the cytosol or nucleus in the colorectal patient cohort (Fig. 3A). In addition, there were no differences in survival for the subgroup with low nuclear $CysLT_1R$ expression (Fig. 3B). However, the subgroup of patients with high nuclear $CysLT_1R$ expression and high nuclear β-catenin expression had significantly reduced survival rates (Fig. 3C).

Table 3 – Univariate and Multivariate Cox proportional hazard model of risk of death. According to cysteinyl leukotriene receptors, adjusted for traditional clinico-pathological factors.

Factor	HR	95% CI	*p*-Value
Univariate analysis			
Gender			
Male	1*		
Female	1.19	0.830–1.70	0.346
Age			
⩽69	1*		
>69	1.03	0.72–1.49	0.864
Tumour localisation			
Proximal	1*		
Distal	0.522	0.347–0.784	0.002
Dukes' stage			
A	1*		
B	3.56	1.67–7.62	0.001
C	6.21	2.99–12.89	<0.001
Differentiation			
High	1*		
Low	0.691	0.282–1.691	0.419
$CysLT_1R$ nucleus			
Low	1*		
High	2.26	1.49–3.43	<0.001
$CysLT_1R$ cytosol			
Low	1*		
High	1.48	0.997–2.19	0.052
$CysLT_2R$ nucleus			
Low	1*		
High	0.552	0.364–0.836	0.005
β-Catenin nucleus			
Low	1*		
High	0.894	0.586–1.36	0.602
β-Catenin cytosol			
Low	1*		
High	1.21	0.816–1.80	0.338
Multivariate analysis			
Tumour localisation			
Proximal	1*		
Distal	0.654	0.367–1.16	0.149
Dukes' stage			
A	1*		
B	2.67	1.00–7.10	0.049
C	6.34	2.49–16.2	<0.001
$CysLT_1R$ nucleus			
Low	1*		
High	1.93	1.06–3.49	0.031
$CysLT_2R$ nucleus			
Low	1*		
High	0.864	0.508–1.47	0.591

HR = hazard ratio and CI = confidence interval.

* Reference group. Only significant factors (univariate $p < 0.05$) were included in the multivariate analysis,

4. Discussion

Colorectal cancer is very well characterised, both clinically and on a molecular level. However, there are no quality prognostic markers in use, clinically. Mutations in β-catenin and adenomatous polyposis coli play a key role in colorectal cancers.[30] Even though the importance of β-catenin signalling in colorectal cancer is well accepted, the value of β-catenin immunohistochemistry is still unclear. There are studies showing an association between elevated levels of β-catenin

Table 4 – β-Catenin immunoreactivity versus potential viability factors.

Relative staining	β-Catenin nuclear staining			*p*-Value
	Low	High	Total	
n (%)	231 (74)	81 (26)	312 (100)	
Gender				0.354[b]
Male	140	54	194 (62.2)	
Female	91	27	118 (37.8)	
Age				
Median	68.8	70.4		0.519[c]
Range	33–90	51–89		0.392[a]
⩽59	36	7	43 (13.8)	
60–69	66	27	93 (29.8)	
70-79	99	39	138 (44.2)	
⩾80	26	8	34 (10.9)	
Missing cases: 4			4 (1.3)	
Tumour localisation				0.020[a]
Proximal colon	22	16	38 (12.2)	
Distal colon	14	8	22 (7.1)	
Rectum	195	57	265 (80.7)	
Dukes' stages				0.413[a]
A	48	17	65 (20.8)	
B	92	26	118 (37.8)	
C & D	91	38	129 (41.4)	
β-Catenin cytosol				<0.001[b]
Low	179	34	213 (68.3)	
High	40	46	86 (27.6)	
Missing cases: 13			13 (4.1)	
Bcl-xL				0.740[b]
Low	40	17	57 (18.3)	
High	139	53	192 (61.5)	
Missing cases: 63			63 (20.2)	

Note: Staining (immunoreactivity) indices are shown in relation to immunostaining for nuclear β-catenin.
a Pearson's χ^2 test (two-sided).
b Fisher's exact test (two-sided) (the Phi coefficient gave equivalent *p*-values).
c Mann–Whitney *U*-test (two-sided).

and patient survival, while other studies demonstrate that β-catenin is associated with shorter survival time.[31] In this study, we observed a correlation between nuclear and cytosolic staining for β-catenin in colorectal cancer patients. Lack of cytosolic staining of β-catenin and reduced membranous staining, together with reduced membranous staining for E-cadherin, has been reported to increase the risk of metastatic disease in rectal cancer patients.[24] However, the expression of β-catenin alone did not mediate any prognostic value for predicting survival. However, the subgroup of patients with high nuclear expression of both $CysLT_1R$ and β-catenin exhibited significantly reduced survival expectancy, indicating that a combination of $CysLT_1R$ and β-catenin could be useful as prognostic markers.

A connection between chronic inflammation in the gut and the development of colon cancer has long been established.[32–35] Around 20% of all cancer cases are attributable to infectious agents that promote neoplastic transformation where inflammatory cells play a major role. In addition, immune cells are frequently present within the stromal microenvironment around tumours.[1] Metabolites of AA, namely PGs, are often upregulated in IBD. Moreover, the enzymes converting AA to PGs and LTs, COX and 5-LO, respectively, are upregulated in both inflammation and cancer.[34,35] We have previously found elevated levels of 5-LO and COX-2 in human colorectal carcinomas.[36] These results were confirmed in vitro in colon carcinoma cell lines and in a non-transformed epithelial cell line.[25] 5-LO is responsible for converting AA into LTB_4 or CysLTs. We have previously shown that signalling from the pro-inflammatory mediator LTD_4 increases survival and proliferation of intestinal epithelial cells.[18,36] Those effects were mediated through the high-affinity receptor for LTD_4 $CysLT_1R$.[15] In addition to the increased downstream activity of $CysLT_1R$, increased levels of the receptor itself have been reported in different colon cancer cell lines and cancer tissues.[25] In agreement with this, earlier studies have shown prolonged survival of patients with lower levels of overall $CysLT_1R$ immunostaining.[25] The observation that $CysLT_1R$ is capable of translocation to the nucleus upon LTD_4 stimulation,[29] as well as reported high levels of nuclear $CysLT_1R$ staining in human colon cancer specimens,[29] motivated us test the value of subcellular localisation of $CysLT_1R$ as a potential prognostic marker. Interestingly, nuclear $CysLT_1R$ staining is significantly associated with survival, and even more strongly so than total $CysLT_1R$ staining. Multivariate prognostic analysis according to the Cox proportional

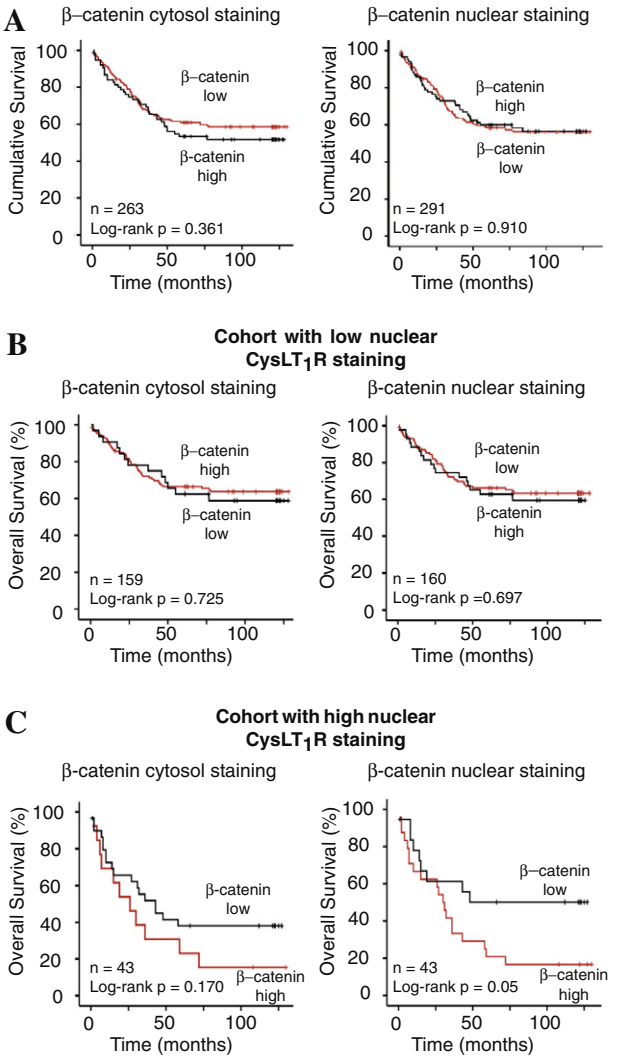

Fig. 3 – Patients with high nuclear CysLT$_1$R and low nuclear β-catenin expression have a better prognosis. (A) Kaplan–Meier survival curves considering cytosolic β-catenin expression (left) and nuclear β-catenin expression (right). (B) Survival curves with cytosolic β-catenin expression (left) and nuclear β-catenin expression (right) in the patient cohort with low nuclear CysLT$_1$R expression. (C) Survival curves with cytosolic β-catenin expression (left) and nuclear β-catenin expression (right) in the patient cohort with high nuclear CysLT$_1$R expression. Patient survival is shown in months and percentage survival for each group was compared using the log-rank test (pooled over strata), missing values (A) *n* = 66 and 38; (B) *n* = 39 and 30; and (C) *n* = 12 and 12.

hazard model confirmed the value of nuclear CysLT$_1$R as an independent prognostic marker.

Our observation that increased levels of CysLT$_1$R in the nucleus are associated with elevated levels of nuclear β-catenin in human colorectal cancer specimens are in line with our previous observation that LTD$_4$ via CysLT$_1$R initiates nuclear translocation of β-catenin, similar to classical Wnt signalling.[17]

The theory that CysLT$_2$R has a protective role in cancer progression is strengthened in this study. Patients exhibiting high nuclear expression of CysLT$_2$R have better survival expectancy than patients with low expression of CysLT$_2$R. The balance between the expressions of the two CysLT receptors seems to influence cancer progression or at least the survival expectancies of colorectal cancer patients. This is demonstrated by the subgroup of patients with high expressions of CysLT$_2$R and low expressions of CysLT$_1$R, which has the best survival prognosis, and the subgroup of patients with high CysLT$_1$R expression and low CysLT$_2$R, which has the worst prognosis.

This study provides additional evidence linking inflammation to the development of cancer, and evidence that the balance between the nuclear expressions of CysLT receptors is important in predicting the survival expectancy of colorectal cancer patients.

Conflict of interest statement

None declared.

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