Personalized Medicine and Imaging

Clinical
Cancer
Research

The Prognostic and Predictive Value of Soluble Type IV Collagen in Colorectal Cancer: A Retrospective Multicenter Study

Hans Christian Rolff[1,2,3], Ib Jarle Christensen[4], Ben Vainer[5], Lars Bo Svendsen[2], Rikke Løvendahl Eefsen[6], Michael Wilhelmsen[4], Ida Katrine Lund[1,3], Gunilla Høyer-Hansen[1,3], Hans Jørgen Nielsen[4], and Martin Illemann[1,3], For the Danish Collaborative Research Group on Colorectal Cancer

Abstract

Purpose: To investigate the prognostic and predictive biomarker value of type IV collagen in colorectal cancer.

Experimental Design: Retrospective evaluation of two independent cohorts of patients with colorectal cancer included prospectively in 2004–2005 (training set) and 2006–2008 (validation set). Plasma samples were available from 297 (training set) and 482 (validation set) patients. Type IV collagen determinations were performed using an ELISA. From the training set, 222 tumors were available for IHC. Clinical and follow-up data were retrieved from patient files and national registries.

Results: High levels of type IV collagen showed independent prognostic significance in both cohorts with hazard ratios (HRs; for a one-unit change on the log base 2 scale) of 2.25 [95% confidence intervals (CIs), 1.78–2.84; $P < 0.0001$] and 2.24 (95% CI, 1.75–2.86; $P < 0.0001$) for the training and validation set, respectively. The prognostic impact was present both in patients with metastatic and nonmetastatic disease. The predictive value of the marker was investigated in stage II and III patients. In the training set, type IV collagen was prognostic both in the subsets of patients receiving and not receiving adjuvant antineoplastic therapy. However, in the validation set, the prognostic effect of the marker vanished when looking at patients who received adjuvant antineoplatic therapy (HR 0.90; 95% CI, 0.42–1.93) but was still present in the group not receiving adjuvant chemotherapy (HR 2.88; 95% CI, 1.98–4.21).

Conclusions: The results indicate clinical validity of type IV collagen as a prognostic biomarker in colorectal cancer, although the suggested predictive role of the marker should be validated.

Clin Cancer Res; 22(10); 2427–34. ©2015 AACR.

Introduction

Colorectal cancer is the third most frequently diagnosed cancer in the Western world (1). Introduction of adjuvant- and targeted antineoplastic therapy (2–4) and advances within radiotherapy and surgery (5–8) have caused a 27% improvement in the 5-year survival rates from 1975 to 2007 (9). New prognostic and predictive biomarkers enabling personalized treatment are intensively studied to improve the survival of patients with colorectal cancer. At present, the only blood-derived biomarker in clinical use is carcinoembronic antigen (CEA), which is used in monitoring oncologic treatment response and to detect recurrence (10). No blood-derived marker is utilized to determine treatment and/or follow-up regimen. Consequently, the treatment is primarily based on disease stage and performance status of the patient (11). There is therefore a great need to identify and validate easily accessible biomarkers enabling identification of patients at high and low risk of early death, independently of disease stage, as this would enable an individualized treatment and follow-up. The focus of this study was to evaluate type IV collagen fragments in blood samples from patients with colorectal cancer in this context.

The main focus of biomarker research has been to identify cancer cell–derived biomarkers. With the recognized tumor–stroma interaction, biomarkers derived from stromal cells have proved to be new and promising biomarker candidates (12). Type IV collagen is a critical component of the epi- and endothelial basement membranes and is synthesized by stromal cells (13). The type IV collagen molecule is a combination of three α-chains out of six different isoforms α1 to α6. Each α-chain consists of an amino terminal 7S-domain, a long central triple-helical region and a carboxy-terminal noncollagenous domain—NC1 domain (14). An increase in the levels of circulating type IV collagen fragments were reported to be associated with the presence of malignant disease in patients with breast cancer (15), presence of liver metastases in colorectal cancer (16), and to be associated

---

[1]The Finsen Laboratory, Rigshospitalet, Copenhagen, Denmark. [2]The Department of Surgical Gastroenterology and Transplantation, Rigshospitalet, Copenhagen, Denmark. [3]Biotech Research Innovation Center–BRIC, University of Copenhagen, Copenhagen, Denmark. [4]Department of Surgical Gastroenterology, Hvidovre Hospital, Hvidovre, Denmark. [5]Department of Pathology, Rigshospitalet, Copenhagen, Denmark. [6]Department of Oncology, Rigshospitalet, Copenhagen, Denmark.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Corresponding Author: Hans Christian Rolff, Department of Surgical Gastroenterology and Transplantation, Rigshospitalet, Blegdamsvej 9, Copenhagen 2100, Denmark. Phone: +45-2989-3883; Fax: +45-3545-6511; E-mail: hrol0008@regionh.dk

doi: 10.1158/1078-0432.CCR-15-2342

Translational Relevance

There is a great need for new noninvasive biomarkers in colorectal cancer, to solve the clinical question of which patients to offer adjuvant antineoplastic therapy. Translation of biomarkers from basic research into the clinic has been very limited in colorectal cancer. We have investigated a novel biomarker, type IV collagen fragments, in plasma samples from two independent colorectal cancer cohorts (test and validation sets) with prospective nationwide multicenter patient inclusion. High levels of type IV collagen fragments were significantly associated with poor overall survival, thus providing strong prognostic information for patients with colorectal cancer. Interestingly, the levels of type IV collagen fragments in plasma may also predict which patients will benefit from adjuvant antineoplastic treatment. Type IV collagen fragments in plasma may in the future change the treatment of patients with colorectal cancer and aid implementation of the personalized medicine principle to improve the survival of these individuals.

with poor prognosis in patients with pancreatic cancer (17). The proposed mechanism for the increase in circulating type IV collagen concentration was tumor-induced tissue remodeling through desmoplasia and proteolysis (17). The desmoplastic stromal response to tumor formation is a common tumor feature and denotes the formation of collagen, formed as a result of recruitment and activation of stromal cells such as fibroblasts (18). Proteolysis is required for the degradation of the extracellular matrix including the basement membrane, where type IV collagen is located. Proteolysis of type IV collagen is primarily carried out by matrix metalloproteases (MMP) 2 and 9, which are expressed by cancer-associated myofibroblasts and tumor-associated macrophages, respectively (19, 20). MMPs can be activated by other protease systems such as the plasminogen activation system (21). It is our hypothesis that the level of circulating type IV collagen fragments is a marker of tissue remodeling hereby reflecting disease activity.

The primary aim of this study was to investigate the prognostic and predictive biomarker value of type IV collagen fragments in colorectal cancer and to evaluate the association between circulating type IV collagen fragments levels with the tumor tissue expression of type IV collagen.

Materials and Methods

Patients

Training set. Subjects were included from November 1st, 2003, to December 31st, 2005. Those eligible for inclusion were adults (18+ years old) referred for large bowel endoscopy due to symptoms of colorectal cancer. The study was a nationwide multicenter study. A total of 4,509 subjects met the initial inclusion criteria prior to endoscopy (22). Of these, endoscopy identified 298 patients with colorectal cancer, and blood samples from these patients were included in this study as the training set. Sufficient plasma volume for measurement was available from 297 of the patients. These plasma samples have previously been used for biomarker studies of tissue inhibitor of metalloproteinases-1 (TIMP-1; ref. 23), CEA (23), intact and cleaved forms of the soluble urokinase-type plasminogen activator receptor (uPAR; ref. 22), human cartilage-glycoprotein 39 (YKL-40; ref. 24), and Cathepsin X (25). Tumor tissue from 244 patients was available and the prognostic value of cell surface bound uPAR has previously been determined by IHC (19).

Validation set. The validation set consisted of patients referred consecutively with a confirmed diagnosis of primary colorectal cancer (all stages). This cohort of 524 patients was also included at multiple national centers from January 2006 to April 2008. Tumor tissue was not available from the validation set. Sufficient plasma samples were available from 482 of the patients.

For both cohorts, patient data regarding demography, disease stage, tumor localization, adjuvant antineoplastic therapy, and survival were retrieved from patient files and national registries such as the National Pathology Registry, the Cancer Registry, and the National Patient Registry.

All patients were treated according to the existing international guidelines at the time of inclusion, thus treatment was not influenced by the type IV collagen measurements.

Ethics

Use of the patient material was approved by the Regional Ethics Committee (training set: KF 01-080/03 and validation set: KF 01-164/03) and the Danish Data Protection Agency (training set: 2003-41-3312 and validation set: KF 2008-41-2252). The study was carried out in accordance with the Helsinki II Declaration.

Sample characteristics

All blood samples were collected according to a prespecified standard operating procedure (SOP; ref. 26). All patients had blood drawn in endotoxin-free, DNase- and RNase-free EDTA, citrate, and serum tubes (Becton, Dickinson and Company). Citrate plasma samples were used for type IV collagen determinations. Samples were centrifuged at 2,500 × *g* for 10 minutes at 4°C. The supernatant closest to the buffy coat (0.5 mL) was left untouched to avoid contamination. The citrated plasma samples were then stored at −80°C under constant electronic surveillance (26).

Tumor tissue specimens for immunohistochemical analyses from the training set were all formalin fixed and paraffin embedded (FFPE).

Type IV collagen determinations

A commercially available enzyme-linked immunoassay (ELISA; EKF Diagnostics, cat. no. BIO82) was used for determination of circulating type IV collagen fragments (α1-chain fragments), which for the remainder of this paper will be referred to just as type IV collagen. The ELISA had been developed and validated for measurements of serum samples, and have been used in previously published studies (15–17, 27–29). Prior to use, we validated the assay for use of citrated plasma samples (Supplementary Data, Appendix S2). The assay performance revealed inter- and intra-assay coefficient of variations below 10%. All determinations were performed blinded with duplicates for each sample and internal control samples on each plate.

Type IV collagen expression in tissue samples

Antibodies. A polyclonal antibody (pAb) against type IV collagen was purchased from Fitzgerald Industries International (cat. no.

20R-CR024). EnVision Horseradish Peroxidase (K4003) was purchased from Dako.

Immunoperoxidase staining. From the FFPE tissue samples, 3-μm thick sections were deparaffinized with xylene and hydrated through ethanol/water solutions. Antigen retrieval were performed by pretreatment with Proteinase K (5 μg/μL) in a Proteinase K buffer (0.5 mmol/L Tris-HCl, 0.5 mmol/L EDTA, pH 8.0) at 37°C for 15 minutes. Endogenous peroxidase activity was blocked by incubation in 1% H_2O_2 for 15 minutes. The sections were washed in Tris-buffered saline (TBS, 50 mmol/L Tris-HCl, 150 mmol/L NaCl) containing 0.5% Triton X-100 (TBS-T), and then mounted in Shandon racks with immunostaining cover plates (Thermo Shandon) for further incubations. The primary antibody was diluted 1:100 in antibody diluent with background-reducing components (S3022, Dako), resulting in a final concentration of 10 μg/mL. The sections were incubated with the primary antibody overnight at 4°C. After incubation, the primary antibody was detected with EnVision Rabbit for 45 minutes. The sections were then treated with NovaRed (Vector Laboratories) for 9 minutes. Each incubation step was followed by washes in TBS-T. Counterstaining was performed using 150 μL of Mayer hematoxylin before the section were dehydrated with ethanol solutions and mounted with pertex using a Dako CoverSlipper.

The level of type IV collagen expression were semiquantitatively assessed in accordance with the scoring system suggested by Nyström and colleagues (30).

Hematoxylin and eosin staining. From the FFPE tissue samples, 3-μm thick sections were deparaffinized with xylene and hydrated through ethanol/water solutions. The sections were placed in Mayer hematoxylin (Sakura Finetek, cat. no. 8710) for 5 minutes followed by washing under running tap water for 5 minutes. The sections were then placed in Eosin 2% (Sakura Finetek, cat. no. 8702) for 5 minutes followed by 1 minute of washing under running tap water. The sections were dehydrated in ethanol solutions and mounted with pertex using a CoverSlipper (Dako).

Study design

All patients were included prospectively, but analyzed retrospectively in accordance with the REMARK guidelines (31). The median follow up periods, calculated by the reverse Kaplan–Meier method, were 56 months (minimum 43 months) and 97 months (minimum 73 months) for the training and validation set, respectively. The primary endpoint for the evaluation of biomarker value of type IV collagen was death of all causes.

Statistical analysis

Descriptive statistics for continuous variables are presented with median and range. Comparisons of patient characteristics were done by the Wilcoxon rank sum test and the χ^2 test of independence. The Spearman rank correlation coefficient was used as a measure of association between variables. Overall survival analyses were done with the Cox proportional hazards model and the results are presented by the HR with 95% confidence intervals (CI). A test for an interaction between adjuvant antineoplastic therapy and the type IV collagen marker was prespecified. The continuous covariate type IV collagen has been scored as log-transformed variables (base 2, meaning that the HRs represent a two-fold difference in type IV collagen concentration). Model validation of the proportional hazards assumption and the linearity of continuous covariates were done using cumulative sums of martingale residuals (32). The model for survival was developed in the training set and the chosen model covariates were then used in the validation set. The chosen covariates were already well established clinically relevant variables such as demographic data (age, gender), localization, disease stage, and adjuvant treatment.

Kaplan–Meier estimates of survival probabilities were calculated for the biomarkers using their respective tertiles as cut-off points. Overall survival was compared using the log-rank test. All calculations were performed using SAS (v9.3, SAS Institute).

Results

Baseline patient characteristics

The baseline patient characteristics of the training and validation set are shown in Table 1. The table shows that the patients in the validation set were older (median age 70 years vs. 68 years, $P = 0.013$), had a higher proportion of rectal cancers (46.5% vs. 37.1%, $P = 0.017$), contained fewer patients with stage IV disease (11% vs. 24%, $P < 0.0001$), and the stage III patients received adjuvant antineoplastic therapy less frequently than the patients in the training cohort (30% vs. 50%, $P = 0.003$). Of the patients receiving adjuvant treatment 87% in the validation set received a fluoropyrimidine-based regimen, either in the form of intravenous 5-fluorouracil (5-FU) 50% or Xeloda 37%. This number was 94% in the validation cohort (intravenous 5-FU 65% and Xeloda 29%). Thirteen percent and 6% received other adjuvant antineoplastic treatments in the training and validation set, respectively.

Type IV collagen in plasma samples

Both the uni- and multivariate analyses (Table 2) showed that type IV collagen was strongly associated to survival. The HRs of the

Table 1. Baseline characteristics

	Training set	Validation set	P
Number of patients	297	482	
Patient demographics			
Median age (min–max)	70 (33–93)	68 (29–95)	0.013
Male (number, %)	179 (60%)	266 (55%)	0.16
Localization			
Colon	185 (62%)	258 (54%)	
Rectum	112 (38%)	224 (46%)	0.017
Stage			
Stage I	47 (16%)	75 (16%)	
Stage II	87 (29%)	168 (35%)	
Stage III	74 (25%)	158 (33%)	
Stage IV	72 (24%)	51 (11%)	<0.0001
Unstaged	17 (6%)	30 (6%)	
Type IV collagen concentration			
All patients (median)	88 ng/mL[a]	87 ng/mL	
Adjuvant antineoplastic therapy			
Stage II	11 (13%)	14 (8%)	0.27
Stage III	37 (50%)	47 (30%)	0.003

NOTE: Baseline characteristics of the two cohorts regarding demography (age and gender), tumor features (stage and localization), median concentration of the marker, and whether or not patients in stages II and III received adjuvant antineoplastic therapy of any sort.

[a] Concentration adjusted according to batch variation (Supplementary Data, Appendix S3).

Table 2. Survival analyses

	Training set		Validation set	
	HR (95% CI)	*P*	HR (95% CI)	*P*
		Univariate		
Type IV collagen				
Continuous	2.83 (2.27–3.53)	<0.0001	2.37 (1.88–2.99)	<0.0001
		Multivariate		
Age				
Per 10 years	1.32 (1.12–1.55)	0.0008	1.41 (1.23–1.62)	<0.0001
Gender				
Female vs. male	0.66 (0.46–0.93)	0.017	0.83 (0.62–1.1)	0.18
Stage				
Stage II vs. stage I	1.11 (0.57–2.15)	0.76	2.04 (1.15–3.61)	0.014
Stage III vs. stage I	2.41 (1.27–4.59)	0.007	2.77 (1.58–4.85)	0.0004
Stage IV vs. stage I	5.39 (2.87–10.1)	<0.0001	8.67 (4.73–15.89)	<0.0001
Localization				
Rectal vs. colon	0.77 (0.53–1.1)	0.14	1.09 (0.816–1.455)	0.56
Type IV collagen				
Continuous	2.25 (1.78–2.84)	<0.0001	2.24 (1.75–2.86)	<0.0001

NOTE: Uni- and multivariate survival analyses. All HRs for type IV collagen concentrations were based on a continuous scale reflecting the increase in mortality rate with a doubling of the concentration of the marker.

univariate analyses were 2.83 (95% CI, 2.27–3.53, $P < 0.0001$) and 2.37 (95% CI, 1.88–2.99, $P < 0.0001$) for the training and validation set, respectively.

The variables included in the multivariate analyses were age, gender, stage, and localization of tumor, and in these multivariate analyses the prognostic significance of type IV collagen was also shown as an independent prognostic parameter in both the training and the validation set with comparable HRs of 2.25 (95% CI, 1.78–2.84, $P < 0.0001$) and 2.24 (95% CI, 1.75–2.86, $P < 0.0001$), respectively.

The Kaplan–Meier survival plots (Fig. 1) show that soluble type IV collagen was a prognostic marker among both the nonmetastatic (stages I–III) and the metastatic patients (stage IV).

By specific evaluation of stage II and III patients, the effect of adjuvant antineoplastic therapy was assessed (Table 3). In the training set, no significant statistical interaction ($P = 0.064$) between type IV collagen and adjuvant antineoplastic therapy was shown, on the contrary, a strong interaction ($P = 0.0068$) was observed in the validation set. This interaction was included in the multivariate analyses. The marker had prognostic significance on both the patients receiving (HR 3.36; 95% CI, 1.06–10.61) and those not receiving adjuvant antineoplastic therapy (HR 2.84; 95% CI, 1.44–5.59) in the training set. In the validation set, the prognostic effect of the marker was absent in the subpopulation receiving antineoplastic therapy (HR 0.9; 95% CI, 0.42–1.93), but still present in the population not receiving antineoplastic therapy (HR 2.88; 95% CI, 1.98–4.21).

Type IV collagen expression in tumor tissue

Because of the promising results from the plasma samples, we continued to analyze the type IV collagen expression in tumor tissue samples. We had access to 244 tissue samples from the training set, of which 222 had representative tumor tissue suitable for quantification.

For all available specimens, H&E-staining and a type IV collagen immunostaining were performed. The stainings are shown in Fig. 2 and revealed an increase in type IV collagen expression upon carcinogenesis. This increase was present both at the invasive front and in the tumor core, but more pronounced in the latter localization.

The collagen expression was scored semiquantitatively as 0 = no expression, 1 = moderate expression, and 2 = high expression according to the scoring system proposed by Nyström and colleagues (30). The scoring was performed at both the tumor core and in the tumor–stroma interface and the two values were summed, resulting in scores ranging from 0 to 4. 11.3% of the patients had a score of 0; 34.7% had a score of 1, 21.2% had a score of 2; 12.6% had a score of 3; and 20.3% of patients had a score of 4.

When investigating the prognostic impact of the type IV collagen tissue expression, no significant association between expression and survival was found ($P = 0.8$). In addition, an association between the tissue expression and the plasma levels could not be shown ($r = -0.10$, $P = 0.12$).

Discussion

The presented results show that plasma type IV collagen is an independent prognostic biomarker for patients with colorectal cancer evaluated in two independent cohorts with prospective patient inclusion. Because of the experimental design with the use of a validation cohort, this article reaches level of evidence II as defined by Simon and colleagues (33).

The two cohorts differed regarding age distribution, stage distribution, and localization of the primary tumor lesion. In spite of these differences, the prognostic significance was similar in the two cohorts showing the robustness of the marker.

There is a need for easily accessible biomarkers to identify patients with poor prognosis, thereby allowing for intensified adjuvant treatment and follow-up with the aim to improve the survival. The only soluble biomarker currently in clinical use is CEA (10). The prognostic effect of CEA has previously been determined on the training set (23). The current results show higher HR values for type IV collagen than that previously reported for CEA. This indicates that type IV collagen may have an increased ability to identify patients with poor prognosis compared with CEA.

Interestingly, the prognostic significance was present both in the nonmetastatic and the metastatic setting (Fig. 1), with the greatest separation the curves in the stage IV group. This

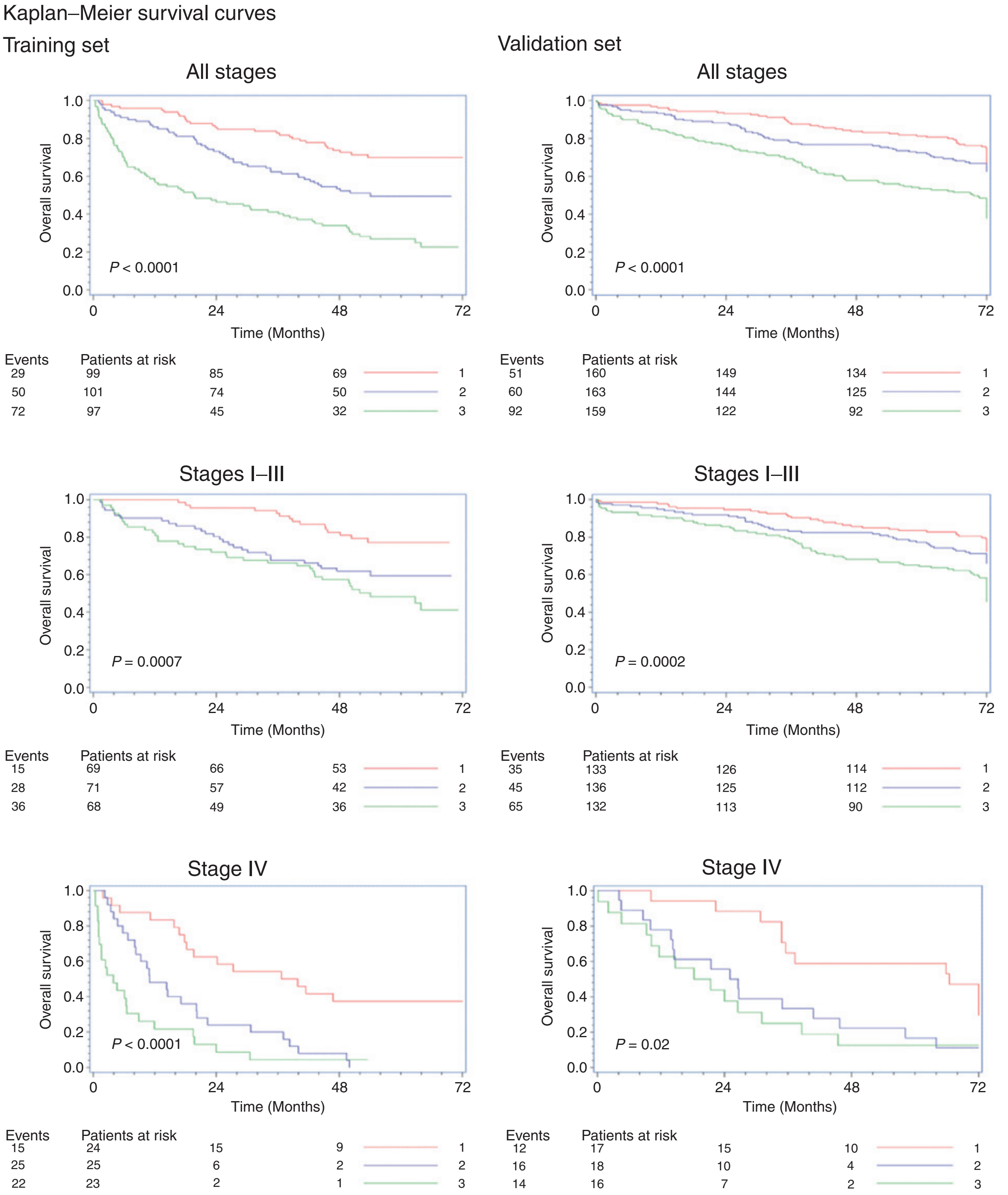

Figure 1.

Kaplan–Meier survival curves for patients with colorectal cancer grouped by the tertiles of their type IV collagen concentrations. Green, blue, and red lines represents the high-, medium-, and low-concentration tertiles, respectively. The number of patients at risk at times 0, 24, and 48 months in each stratum are shown below the axes with the number of deaths shown to the left.

may be due to the fact that the role of tumor biology is most pronounced in this stage as there is less possibility of surgical removal of the tumor.

The evaluation of a potential predictive biomarker value in the present cohorts was not entirely sufficient due to the fact that relatively few patients received adjuvant antineoplastic therapy.

Table 3. Survival analyses including adjuvant antineoplastic therapy on patients with stage II and III colorectal cancer

	Training set		Validation set	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Number of patients	161		326	
Age				
Per 10 years	1.43 (1.11–1.84)	0.0057	1.59 (1.32–1.92)	<0.0001
Gender				
Female vs. male	0.64 (0.38–1.09)	0.099	0.69 (0.47–1.002)	0.051
Stage				
Stage II vs. stage III	0.45 (0.26–0.80)	0.0058	0.82 (0.55–1.20)	0.30
Localization				
Colon vs. rectal	1.29 (0.72–2.31)	0.40	0.70 (0.48–1.03)	0.068
Type IV collagen and adjuvant antineoplastic therapy				
No adjuvant antineoplastic therapy	2.84 (1.44–5.59) (*n* = 113)		2.88 (1.98–4.21) (*n* = 265)	
Adjuvant antineoplastic therapy	3.36 (1.06–10.61) (*n* = 48)	0.063[a]	0.90 (0.42–1.93) (*n* = 61)	0.0068[a]

NOTE: Multivariate analyses for patients in stages II and III. The variables include age, gender, stage, localization, interaction between type IV collagen, and adjuvant antineoplastic therapy.

[a] Refers to the interaction between type IV collagen and adjuvant antineoplastic therapy.

The reason for this was that the inclusion periods, which were 2003–2005 and 2006–2008 for the training and validation sets, respectively. Adjuvant treatment for stage III colon cancer was introduced in 2005, and adjuvant treatment for high-risk colon cancer stage II, rectal cancer stage III and selected high-risk rectal cancer was introduced in 2009 (34). Relatively few patients received adjuvant antineoplastic therapy, with minor differences between the training and validation set, which is why the variable was treated as a binary covariate in the statistical analyses. Our results indicate that type IV collagen is a predictive biomarker, when looking only at stage II and III patients, even though our findings were not consistent between the two cohorts. The prognostic significance was present in all patients in the training set independent of antineoplastic therapy. However, in the validation cohort the prognostic significance of the marker was not present in the subpopulation of patients receiving adjuvant antineoplastic therapy. This suggests that the administration of adjuvant antineoplastic therapy levels out the poor prognosis for patients with a high type IV collagen plasma concentration. This particular aspect of the applicability of the marker must be evaluated further.

The plasma concentrations of type IV collagen in the training set were adjusted due to batch variation in the commercial ELISAs, resulting in a parallel shift of the standard curves between the two cohorts. The adjustment is further explained in Supplementary Data, Appendix S3. Thus, even if unadjusted, the batch variation did not influence the results, as the type IV collagen plasma concentration was treated as a continuous variable in the statistical analyses and no cut points were applied. In addition, samples from the two cohorts were determined and statistically analyzed independently.

The type IV collagen analyte detected in the assay represents the 7S domain and the triple-helical region of the α1 chain (27, 29). As mentioned previously, the exact mechanism underlying the formation of the analyte is largely unknown. Previous studies have suggested that proteolysis and desmoplasia as a result of tissue remodeling are the responsible mechanisms (15, 17). Patients with fibrotic and cirrhotic liver disorders also have increased levels of circulating type IV collagen (27, 29), indicating that the mechanism for type IV collagen release is not exclusively cancer specific.

The NC1 domain of the α1-chain is not detected in the present type IV collagen assay. This part of the type IV collagen molecule contains the molecule Arresten, which has been found to have an antiangiogenic and antitumor effect (35). Because of the complex and highly dynamic biology of the tumor microenvironment with substantial cross-talk and redundancy between different cellular and pericellular processes, it is not

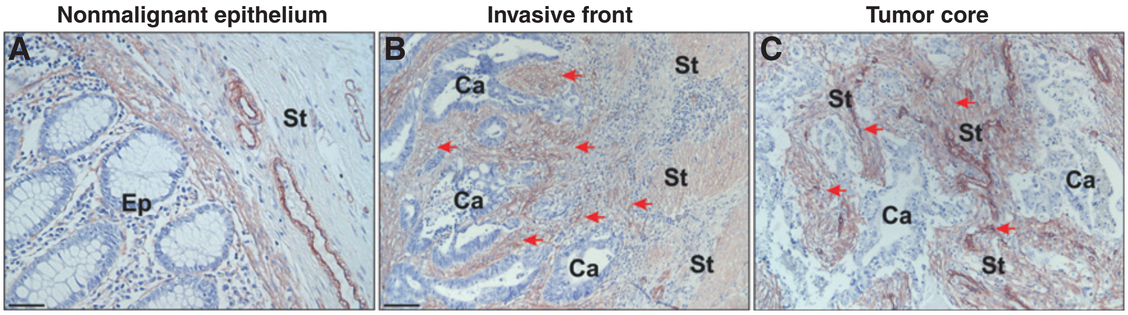

Figure 2.

Type IV and total collagen expression in normal and malignant colonic tissue. Adjacent sections of colorectal adenocarcinomas were subject to type IV collagen-IHC using a pAb against type IV collagen (A–C). The type IV collagen stainings were visualized with NovaRed. Nonmalignant epithelium is shown in A, the invasive front of the cancer is shown in B, and the tumor core is shown in C. In the nonmalignant epithelium (A), there was no type IV collagen expression within the epithelial (Ep) lining. In the invasive front (B) and in the tumor core (C), we observed an increased expression/accumulation of type IV collagen in the stroma (St) surrounding the cancer cells (Ca) (brown staining highlighted with red arrows in B and C). Bar in A (representing picture A), approximately 50 μm. Bar in B (representing pictures B and C), approximately 100 μm.

unlikely that the isolated biologic properties of Arresten are overshadowed by other processes such as continuous matrix degradation and tissue remodeling.

Type I collagen has also been shown to play a role in colorectal cancer carcinogenesis as the degraded telopeptide of type I collagen was correlated to the presence of colorectal cancer and also associated with stage and prognosis (36, 37). The biologic role of the overall collagen composition and metabolism in the tumor microenvironment is a relatively new research field which is gaining further focus, due to these recent interesting findings.

The tissue stainings for type IV collagen did not provide any significant prognostic information. This finding was unexpected as other studies had shown increased progression and poor survival in cases with high type IV collagen tissue expression (16, 17, 30). Our data were the result of a blinded evaluation of the type IV collagen expression. We did, however, see a general upregulation of type IV collagen expression upon carcinogenesis. The lack of prognostic information in our study may be a result of tumor heterogeneity as well as primary antibody and the scoring model. We have previously analyzed uPAR expression in the same samples. In that case, uPAR-positive macrophages in the tumor core were significantly associated to overall survival (19). It could therefore be suggested that it provides more information to identify members of protease systems responsible for the proteolytic activity in the tumor stroma than it is to identify the original substrate molecules such as type IV collagen.

Because of the complexity of the tumor microenvironment it is unlikely that a single biomarker is sufficient in describing the complex biologic features of the tumor and its microenvironment. A panel of valid biomarkers reflecting the different hallmarks of cancer (38) could prove useful for patient-tailored treatments based on molecular tumor characteristics in addition to already established clinicopathologic tumor features.

As the hypothesized processes responsible for the formation of the type IV collagen analyte are general features for all epithelial malignant tumors, it is likely that type IV collagen could act as a biomarker in other tumor types than colorectal cancer. Research in other biomarker settings than the prognostic and predictive, including selection, monitoring, and surrogate settings could also offer possible new applications that must be included in future explorative studies. Indeed, type IV collagen has been found to correlate to disease activity as removal of colorectal liver metastases lead to a decrease in the serum type IV collagen concentration. In addition, recurrence of disease was associated with an increase in the type IV collagen concentration (16).

Even though the underlying biologic mechanisms behind the formation of the type IV collagen analyte remain to be completely clarified, the validated results in this study proves clinical validity of determining type IV collagen in plasma samples from patients with colorectal cancer. In spite of the convincing clinical validity there are still major tasks to be undertaken prior to an eventual clinical implementation. The preanalytical aspects of the assay need to be further investigated to establish well-documented references regarding sampling, storage, handling, and reference values. Another aspect is that due to changes in the standard treatment of patients with colorectal cancer during the inclusion period (2004–2008) and the introduction of new therapies, it is difficult to translate the findings of this study directly in to the present clinical setting. Therefore, prospective studies with recent patient inclusion focusing on the clinical utility are needed to assess if implementation of the assay to the everyday clinical routine when treating patients with colorectal cancer could offer an improved survival.

Conclusion

The results of this study showed independent prognostic and possibly predictive impact of circulating type IV collagen in citrate plasma samples from patients with colorectal cancer. Further studies are needed to clarify the preanalytical aspects of the biomarker. In addition, sufficiently powered, prospective clinical studies must be performed with focus on confirming the prognostic value and verifying the predictive value in detail.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: H.C. Rolff, I.J. Christensen, B. Vainer, G. Høyer-Hansen, H.J. Nielsen, M. Illemann

Development of methodology: H.C. Rolff, I.J. Christensen, B. Vainer, I.K. Lund, M. Illemann

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): H.C. Rolff, I.J. Christensen, M. Wilhelmsen, G. Høyer-Hansen, H.J. Nielsen, M. Illemann

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): H.C. Rolff, I.J. Christensen, B. Vainer, L.B. Svendsen, I.K. Lund, H.J. Nielsen, M. Illemann

Writing, review, and/or revision of the manuscript: H.C. Rolff, I.J. Christensen, B. Vainer, L.B. Svendsen, R.L. Eefsen, M. Wilhelmsen, I.K. Lund, G. Høyer-Hansen, H.J. Nielsen, M. Illemann

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): H.C. Rolff, I.J. Christensen, B. Vainer, I.K. Lund, M. Illemann

Study supervision: H.C. Rolff, B. Vainer, L.B. Svendsen, G. Høyer-Hansen, H.J. Nielsen, M. Illemann

Acknowledgments

The authors thank the research nurses, secretaries, and technicians at the participating hospital departments and laboratories are thanked for their skillful work.

Grant Support

The work was funded by the Department of Surgical Gastroenterology and Transplantation, Rigshospitalet (Copenhagen, Denmark). The study also received financial support from The Danish Cancer Society, The Kornerup Fund, The Aage and Johanne Louis-Hansen Fund, The Aase and Ejnar Danielsen Fund, The Walter and O. Kristiane Christensen Fund, The Kathrine and Vigo Skovgaard Fund, Den Midtjyske Bladfond, The Agnes and Poul Friis Fund, The Glunz and Jensen Fund, The Sophus and Astrid Jacobsen Fund, The Arvid Nilsson Fund, The Danish Bank Fund, The Johannes Fog Fund, The Eva and Henry Fraenkel Fund, The Hartmann Bros. Fund, The KID Fund, The Henrik Henriksen Fund, The King Christian X's Fund, The Oda and Hans Svenningsen Fund, The Else and Mogens Wedell-Wedellsborg Fund, The Einar Willumsen Fund, The Willy and Ingeborg Reinhard Fund, The Friedrich and Else Boehm Fund, The Toyota Fund Denmark, The IMK Fund, The Danish Medical Research Fund, The Beckett Fund and Hvidovre University Hospital.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received October 1, 2015; revised November 13, 2015; accepted November 30, 2015; published OnlineFirst December 16, 2015.

References

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010;127:2893–917.
2. Andre T, Boni C, Mounedji-Boudiaf L, Navarro M, Tabernero J, Hickish T, et al. Oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment for colon cancer. N Engl J Med 2004;350:2343–51.
3. Giantonio BJ, Catalano PJ, Meropol NJ, O'Dwyer PJ, Mitchell EP, Alberts SR, et al. Bevacizumab in combination with oxaliplatin, fluorouracil, and leucovorin (FOLFOX4) for previously treated metastatic colorectal cancer: results from the Eastern Cooperative Oncology Group Study E3200. J Clin Oncol 2007;25:1539–44.
4. Van Cutsem E, Peeters M, Siena S, Humblet Y, Hendlisz A, Neyns B, et al. Open-label phase III trial of panitumumab plus best supportive care compared with best supportive care alone in patients with chemotherapy-refractory metastatic colorectal cancer. J Clin Oncol 2007; 25:1658–64.
5. Påhlman L, Glimelius B. Improved survival with preoperative radiotherapy in resectable rectal cancer. Swedish Rectal Cancer Trial. N Engl J Med 1997;336:980–7.
6. Bertelsen CA, Neuenschwander AU, Jansen JE, Wilhelmsen M, Kirkegaard-Klitbo A, Tenma JR, et al. Disease-free survival after complete mesocolic excision compared with conventional colon cancer surgery: a retrospective, population-based study. Lancet Oncol 2015;16:161–8.
7. Heald RJ, Moran BJ, Ryall RD, Sexton R, MacFarlane JK. Rectal cancer: the Basingstoke experience of total mesorectal excision, 1978–1997. Arch Surg 1998;133:894–9.
8. MacFarlane JK, Ryall RD, Heald RJ. Mesorectal excision for rectal cancer. Lancet 1993;341:457–60.
9. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin 2012;62:10–29.
10. Duffy MJ, Lamerz R, Haglund C, Nicolini A, Kalousová M, Holubec L, et al. Tumor markers in colorectal cancer, gastric cancer and gastrointestinal stromal cancers: European group on tumor markers 2014 guidelines update. Int J Cancer 2014;134:2513–22.
11. American Joint Commitee on Cancer. AJCC cancer staging manual. 7th ed. New York: Springer; 2009.
12. Sund M, Kalluri R. Tumor stroma derived biomarkers in cancer. Cancer Metastasis Rev 2009;28:177–83.
13. Khoshnoodi J, Pedchenko V, Hudson BG. Mammalian collagen IV. Microsc Res Tech 2008;71:357–70.
14. Kalluri R. Basement membranes: structure, assembly and role in tumour angiogenesis. Nat Rev Cancer 2003;3:422–33.
15. Mazouni C, Arun B, André F, Ayers M, Krishnamurthy S, Wang B, et al. Collagen IV levels are elevated in the serum of patients with primary breast cancer compared to healthy volunteers. Br J Cancer 2008;99:68–71.
16. Nyström H, Naredi P, Hafström L, Sund M. Type IV collagen as a tumour marker for colorectal liver metastases. Eur J Surg Oncol 2011; 37:611–7.
17. Ohlund D, Lundin C, Ardnor B, Oman M, Naredi P, Sund M. Type IV collagen is a tumour stroma-derived biomarker for pancreas cancer. Br J Cancer 2009;101:91–7.
18. Kalluri R, Zeisberg M. Fibroblasts in cancer. Nat Rev Cancer 2006;6: 392–401.
19. Illemann M, Laerum OD, Hasselby JP, Thurison T, Høyer-Hansen G, Nielsen HJ, et al. Urokinase-type plasminogen activator receptor (uPAR) on tumor-associated macrophages is a marker of poor prognosis in colorectal cancer. Cancer Med 2014;3:855–64.
20. Illemann M, Bird N, Majeed A, Sehested M, Laerum OD, Lund LR , et al. MMP-9 is differentially expressed in primary human colorectal adenocarcinomas and their metastases. Mol Cancer Res 2006;4:293–302.
21. He Y, Liu XD, Chen ZY, Zhu J, Xiong Y, Li K, et al. Interaction between cancer cells and stromal fibroblasts is required for activation of the uPAR-uPA-MMP-2 cascade in pancreatic cancer metastasis. Clin Cancer Res 2007;13:3115–24.
22. Thurison T, Lomholt AF, Rasch MG, Lund IK, Nielsen HJ, Christensen IJ , et al. A new assay for measurement of the liberated domain I of the urokinase receptor in plasma improves the prediction of survival in colorectal cancer. Clin Chem 2010;56:1636–40.
23. Nielsen HJ, Brunner N, Jorgensen LN, Olsen J, Rahr HB, Thygesen K , et al. Plasma TIMP-1 and CEA in detection of primary colorectal cancer: a prospective, population based study of 4509 high-risk individuals. Scand J Gastroenterol 2011;46:60–9.
24. Johansen JS, Christensen IJ, Jorgensen LN, Olsen J, Rahr HB, Nielsen KT , et al. Serum YKL-40 in risk assessment for colorectal cancer: a prospective study of 4,496 subjects at risk of colorectal cancer. Cancer Epidemiol Biomarkers Prev 2015;24:621–6.
25. Vizin T, Christensen IJ, Wilhelmsen M, Nielsen HJ, Kos J. Prognostic and predictive value of cathepsin X in serum from colorectal cancer patients. BMC Cancer 2014;14:259.
26. Nielsen HJ, Brunner N, Frederiksen C, Lomholt AF, King D, Jorgensen LN, et al. Plasma tissue inhibitor of metalloproteinases-1 (TIMP-1): a novel biological marker in the detection of primary colorectal cancer. Protocol outlines of the Danish-Australian endoscopy study group on colorectal cancer detection. Scand J Gastroenterol 2008;43:242–8.
27. Ikuta Y, Yamada S, Murawaki Y, De- S. Comparison of serum 7s fragment of type IV collagen and serum central triple-helix of type IV collagen for assessment of liver fibrosis in patients with chronic viral liver disease. J Hepatol 1996;24:148–54.
28. Kinoshita J, Fushida S, Harada S, Makino I, Nakamura K, Oyama K, et al. Type IV collagen levels are elevated in the serum of patients with peritoneal dissemination of gastric cancer. Oncol Lett 2010;1:989–94.
29. Obata Ki, Iwata K, Ichida T, Inoue K, Matsumoto E, Muragaki Y, et al. One step sandwich enzyme immunoassay for human type IV collagen using monoclonal antibodies. Clin Chim Acta 1989;181:293–303.
30. Nyström H, Naredi P, Berglund A, Palmqvist R, Tavelin B, Sund M. Liver-metastatic potential of colorectal cancer is related to the stromal composition of the tumour. Anticancer Res 2012;5192:5183–91.
31. McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM. REporting recommendations for tumour MARKer prognostic studies (REMARK). Br J Cancer 2005;93:387–91.
32. Lin DY, Wei JL, Yang Z. Checking the cox model with cumulative sums of martingale-based residuals. Biometrika 1993;80:557–72.
33. Simon RM, Paik S, Hayes DF. Use of archived specimens in evaluation of prognostic and predictive biomarkers. J Natl Cancer Inst 2009;101: 1446–52.
34. DMCG Benchmark Consortium; 2015. Available from: http://dmcg.dk/fileadmin/dmcg.dk/Benchmarking_Consortium/D_Colorektal_07_11__2014_v2.pdf.
35. Nyberg P, Xie L, Sugimoto H, Colorado P, Sund M, Holthaus K, et al. Characterization of the anti-angiogenic properties of arresten, an alpha1-beta1 integrin-dependent collagen-derived tumor suppressor. Exp Cell Res 2008;314:3292–305.
36. Zou X, Feng B, Dong T, Yan G, Tan B, Shen H, et al. Up-regulation of type I collagen during tumorigenesis of colorectal cancer revealed by quantitative proteomic analysis. J Proteomics 2013;94:473–85.
37. Bode MK, Karttunen TJ, Makela J, Risteli L, Risteli J. Type I and III collagens in human colon cancer and diverticulosis. Scand J Gastroenterol 2000;35:747–52.
38. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144:646–74.

American Association for Cancer Research

Clinical Cancer Research

The Prognostic and Predictive Value of Soluble Type IV Collagen in Colorectal Cancer: A Retrospective Multicenter Study

Hans Christian Rolff, Ib Jarle Christensen, Ben Vainer, et al.

Clin Cancer Res 2016;22:2427-2434. Published OnlineFirst December 16, 2015.

Updated version	Access the most recent version of this article at: doi:10.1158/1078-0432.CCR-15-2342
Supplementary Material	Access the most recent supplemental material at: http://clincancerres.aacrjournals.org/content/suppl/2015/12/16/1078-0432.CCR-15-2342.DC1

Cited articles	This article cites 36 articles, 6 of which you can access for free at: http://clincancerres.aacrjournals.org/content/22/10/2427.full#ref-list-1
Citing articles	This article has been cited by 1 HighWire-hosted articles. Access the articles at: http://clincancerres.aacrjournals.org/content/22/10/2427.full#related-urls

E-mail alerts	Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions	To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions	To request permission to re-use all or part of this article, use this link http://clincancerres.aacrjournals.org/content/22/10/2427. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.