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Cancer Stem Cell Gene Variants in CD44 Predict Outcome in Stage II and Stage III Colon Cancer Patients

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Abstract. *Background/Aim: Growing evidence suggests that human cancers are stem cell diseases and recent data support the existence of cancer stem cells (CSCs) in a variety of malignancies, including colon cancer. These CSCs were shown to be capable of initiating tumor development and progression. Several studies have suggested CD133, CD26 and CD44 as markers of tumor-initiating cells of colon cancer. The purpose of the present study was to assess the impact of single-nucleotide polymorphisms (SNPs) in stem cell-related genes on clinical outcome in a large cohort of colon cancer patients with clinical stage II and III. Patients and Methods: Data from 599 consecutive patients with colon cancer stage II and III, treated between 1995 and 2011 at a single centre, were retrospectively evaluated. Genomic DNA was extracted from paraffin-embedded normal tissue distant from the tumor to obtain germline DNA. Allelic distribution of polymorphisms was tested for deviation from Hardy-Weinberg equilibrium using χ^2-test. The association of polymorphisms with time to recurrence (TTR) and overall survival (OS) was analyzed using Kaplan-Meier curves and compared by the log-rank test. Case-wise deletion for missing polymorphisms was used in univariable and multivariable analyses. Results: CD44 rs187115 showed a statistically significant association with TTR; patients carrying at least one G allele had a significant reduced risk of recurrence compared to patients with the homozygous A/A variant (hazard ratio (HR)=0.67, 95% confidence interval (CI)=0.48-0.94, p=0.019). CD44 rs13347 showed a statistically significant association with OS. Patients carrying at least one T allele in rs13347 had a significantly reduced risk of death compared to patients with the homozygous C/C variant (HR=0.61, 95% CI=0.41-0.92, p=0.019). None of the other investigated polymorphisms (CD44 rs187116, CD44 rs7116432, CD44 rs353639, DPP4 rs2268889, DPP4 rs3788979, DPP4 rs7608798 and CD133 rs2240688) were associated with either TTR or OS. Conclusion: Germline variants rs13347 and rs187115 in the stem cell gene CD44 are prognostically relevant in stage II and III colon cancer patients.*

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Colon cancer is the third most common cancer worldwide, likewise affecting male and female patients (1). Nearly half the patients with colon cancer develop synchronous or metachronous metastases. Five-year survival rates of less than 10% in the metastatic setting are responsible for a relatively high mortality rate (2). Moreover, tumor recurrence after curative surgery is still a major problem. Standard treatment for patients with stage III and high-risk stage II colon cancer after curative surgery consists of 5-fluorouracil (5-FU)-based chemotherapy, aiming at reducing the risk of relapse (3). Thus, in non-metastatic disease, 5-year survival rates range between 40-90%, depending on the clinical stage (4). Despite adjuvant treatment, a considerable number of colon cancer patients still develop tumor recurrence or distant metastases. More precise biomarkers are needed to guide adjuvant treatment through patient classification in order to avoid unnecessary chemotherapy and increase outcome of colon cancer patients (5, 6).

It is now widely accepted that multiple factors contribute to the efficacy of chemotherapy and that treatment should be

optimized on an individual case-specific basis. Recent evidence suggests that human cancers are stem cell diseases (7-11). Cancer stem cells (CSCs) possess the ability to self-renew, undergo multi-lineage differentiation and to survive an unfavourable tissue microenvironment (12, 13). Moreover, colon cancer studies have identified CSCs being capable of initiating tumor development (14-16). CD133 (*PROM1*), CD26 (dipeptidyl peptidase 4 - *DDP4*) and *CD44* have been suggested as markers of tumor-initiating cells of colon cancer (7, 13, 16). DPP4 was shown to be involved in cancer-related processes, such as migration, apoptosis, invasion and sensitivity to chemotherapy (17). A decreased or missing DPP4 expression was discovered in various tumor entities. Wesley *et al*. could identify DPP4 as a tumor suppressor, with its down-regulation leading to limited growth control (18). CD133 is a trans-membrane cell-surface glycoprotein and expressed by several cell types including CSCs. It's function is still uncertain; however, high CD133 expression correlates with poor survival in tumors as lung-, prostate- and colon cancer (19). Especially CD44, a major cell adhesion molecule, plays a role in various cellular processes, including migration, cellular binding and regulation of growth and homing of lymphocytes (20). Considering that CD44 promotes several tumorigenic processes, it seems likely that the *CD44* gene harbors functional genetic variants potentially serving as molecular prognostic and/or predictive markers in colon cancer.

In this study, we investigated nine germline polymorphisms in genes that have previously been associated with colon cancer CSCs and aimed at predicting tumor recurrence in 599 patients with stage III and high-risk stage II colon cancer.

Patients and Methods

Eligible patients. Between 1995 and 2011, 801 Caucasian patients with a histopathologically confirmed stage II (n=373) and III (n=428) colon cancer were consecutively recruited at the Division of Clinical Oncology, Department of Medicine, Medical University of Graz, Austria. Clinical stage according to Union for International Cancer Control (UICC) was assessed based on the radiomorphologic presentation at the time of surgery, as well as the resection specimen. From 599 patients, paraffin-embedded normal tissue adjacent to tumor samples was available for germline genetic testing. Two hundred and eight patients underwent surgery only, whilst 391 patients additionally received adjuvant 5-FU-based chemotherapy. All patients were included in a colon cancer surveillance program, suggesting history and physical examination and carcinoembryonic antigen (CEA) determination every 3 months for 3 years, every 6 months at years 4 and 5 and yearly at years 6-10 after surgery. Colonoscopy was performed at year 1 and thereafter every 3-5 years, X-ray of the chest and abdominal ultrasound or computed tomography (CT) scans of chest and abdomen every 3-6 months for the first 5 years and X-ray of the chest and abdominal ultrasound annually from year 6 to10. Patient data was retrospectively ascertained by chart review. This study has been approved by the Institutional Review Board (IRB) of the Medical University of Graz (25-457 ex 12/13).

Isolation of genomic DNA and determination of single-nucleotide polymorphisms (SNPs). Tissue samples were stored at the Biobank of the Medical University of Graz (certified according to EN/ISO 9001:2008). Genomic DNA was extracted from paraffin-embedded normal tissue distant from the tumor to obtain germline DNA. Samples from the resection margins were used after re-evaluation by a board certified pathologist to ensure tumor-free tissue. DNA isolation was performed using the QIAamp DNA mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. Genotypes for CD44 (rs187116 A>G, rs7116432 A>G, rs353639 A>C, rs13347 C>T and rs187115 A>G), DPP4 (rs2268889 A>G, rs3788979 A>G, rs7608798 A>G) and CD133 (rs2240688 A>C) were centrally determined by 5'-exonuclease assay (TaqMan; Thermo Fisher Scientific, Vienna, Austria). Primer and probe sets were designed and manufactured using Applied Biosystems 'Assay-by-Design' custom service (Applera, Vienna, Austria). General TaqMan reaction conditions were according to the manufacturer of the assays. End-point fluorescence was measured in a Lambda Fluoro 320 plus plate reader (MWG Biotech AG, Ebersberg, Germany) using excitation/emission filters of 485/530 and 530/572 nanometers (nm), respectively. The data were exported into Excel format, subsequently analyzed and depicted as scatter plots. In the plots, genotype groups were identified as separate and distinguishable clusters. As a control for consistency of the genotyping method, determination of genotypes was repeated in at least 96 samples. The rules of good laboratory and clinical practice were observed. The investigator analyzing the germline polymorphisms was blinded to the clinical data set.

Statistical analysis. The primary end-point of the study was time to recurrence (TTR), which was defined as the time from date of diagnosis of colon cancer to the date of first tumor recurrence. If a patient's tumor had not recurred, TTR was censored at the time of death or at the last follow-up. The secondary end-point was overall survival (OS), which was defined as the time from date of diagnosis of colon cancer to the date of death from any cause. Allelic distribution of polymorphisms was tested for deviation from Hardy-Weinberg equilibrium using χ^2-test. The true mode of inheritance of the polymorphism tested has not been established yet and we assumed a dominant or recessive genetic model where appropriate. However, we did not find any differences between the dominant and the recessive model; therefore, we subsequently only used the dominant model. The association of polymorphisms with TTR and OS was analyzed using Kaplan-Meier curves and compared by log-rank test. Demographic and clinicopathological features were included in multivariable analysis when a p-value of <0.2 had been achieved in univariable analysis. In a stepwise backward multivariable Cox-regression analysis for TTR, the features age, tumor location, tumor size, number of resected lymph nodes, lymphovascular, vascular and perineural invasion and stage were included. For OS, the features age, tumor side, invasion depth, number of resected lymph nodes, tumor grade, lymphovascular, vascular and perineural invasion, and stage as well as application of adjuvant chemotherapy were included. Hazard ratios (HR) and 95% confidence intervals (CI) were reported. Case-wise deletion for missing polymorphisms was used in univariable and multivariable analyses. A p-value <0.05 was considered to be statistically significant. All analyses were performed using SPSS for Windows (Version 22; SPSS Inc., Chicago, IL, USA).

Table I. *Baseline patients' characteristics and their association with TTR and OS in univariable analysis.*

Parameter	N	%	TTR		OS	
			HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value
Gender						
Male	330	55.1	1 (reference)	0.861	1 (reference)	0.300
Female	269	44.9	0.98 (0.73-1.30)		0.85 (0.63-1.16)	
Age						
in years			1.02 (1.00-1.03)	0.012	1.05 (1.04-1.07)	<0.001
Tumor location						
Left	375	62.6	1 (reference)	0.075	1 (reference)	0.058
Right	224	37.4	1.31 (0.97-1.75)		1.35 (0.99-1.83)	
Lymph node operated						
≤12	84	14.0	1 (reference)	0.868	1 (reference)	0.734
> 12	515	86.0	1.04 (0.69-1.56)		0.93 (0.63-1.39)	
Tumor size						
T1 & T2	35	5.8	1 (reference)	<0.001	1 (reference)	<0.001
T3	434	72.5	2.62 (0.97-7.10)		1.80 (0.74-4.42)	
T4	130	21.7	5.28 (1.81-14.59)		4.22 (1.69-10.56)	
Lymph node involvement						
N0	237	39.6	1 (reference)	<0.001	1 (reference)	<0.001
N1	229	38.2	1.60 (1.09-2.34)		1.41 (0.97-2.06)	
N2	133	22.2	4.17 (2.89-6.03)		2.94 (2.02-4.29)	
Tumor grade						
G1 & G2	418	69.8	1 (reference)	0.279	1 (reference)	0.017
G3	181	30.2	1.19 (0.87-1.61)		1.46 (1.07-1.99)	
Lymphovascular invasion						
No	435	72.6	1 (reference)	0.007	1 (reference)	0.136
Yes	164	27.4	1.51 (1.12-2.05)		1.28 (0.93-1.78)	
Vascular invasion						
No	539	90.0	1 (reference)	<0.001	1 (reference)	<0.001
Yes	60	10.0	2.41 (1.67-3.49)		2.06 (1.38-3.08)	
Perineural invasion						
No	584	97.5	1 (reference)	<0.001	1 (reference)	0.060
Yes	15	2.5	3.84 (2.09-7.27)		2.07 (0.97-4.41)	
Clinical stage						
II	235	39.2	1 (reference)	<0.001	1 (reference)	<0.001
III	364	60.8	2.40 (1.72-3.35)		1.92 (1.38-2.67)	
Adjuvant chemotherapy						
No	208	34.7	1 (reference)	0.236	1 (reference)	0.110
Yes	391	65.3	1.21 (0.88-1.65)		0.78 (0.57-1.06)	

TTR, Time to recurrence; OS, overall survival; HR, hazard ratio; CI, confidence interval.

Results

The baseline characteristics of the 599 patients included in the analysis and their association with TTR and OS are visible in Table I. Median age at the time of diagnosis was 65 years (range=27-95). The genotyping quality control provided a genotype concordance of 99%. Genotyping was successful in at least 71.6% of cases for each polymorphism analyzed (range=71.6-89.3%). In failed cases, genotyping was not successful because of limited quantity and/or quality of extracted genomic DNA. The allelic frequencies for 8 of 9 polymorphisms were within the probability limits of the Hardy-Weinberg equilibrium. For *DPP4*, the rs2268889 allelic frequency was not in the probability limit of Hardy-Weinberg equilibrium (data not shown).

Tumor recurrence was observed in 185 (30.9%) patients with a 5-year recurrence probability of 34.9% (standard deviation (SD)=2.2). Tumors recurred in 46 out of 235 stage II colon cancer patients (19.6%) and in 139 out of 364 stage III colon cancer patients (38.2%). Nine polymorphisms were investigated, with the variant CD44 rs187115 showing a statistically significant association with TTR in univariable analysis. Patients carrying at least one G allele had a significantly reduced risk of recurrence compared to patients

Table II. *SNP rs13347 and SNP rs187115 and their association with TTR and OS in multivariable analysis (dominant model).*

Parameter	SNP rs13347				SNP rs187115			
	TTR		OS		TTR		OS	
	HR(95%CI)	*p*-Value	HR(95%CI)	*p*-Value	HR(95%CI)	*p*-Value	HR(95%CI)	*p*-Value
SNP rs13347					n.d.	n.d.	n.d.	n.d.
C/C	1 (reference)	0.076	0.61 (0.41-0.92)	0.019				
C/T or T/T	0.72 (0.50-1.04)							
SNP rs187115	n.d.	n.d.	n.d.	n.d.				
A/A					1 (reference)	0.019	1 (reference)	0.921
A/G or G/G					0.67 (0.48-0.94)		0.98 (0.68-1.42)	
Age	1.02 (1.01-1.04)	0.012	1.05 (1.03-1.07)	<0.001	1.02 (1.01-1.04)	0.009	1.05 (1.03-1.07)	<0.001
Tumor location	n.d.	n.d.	n.d.	n.d.			n.d.	n.d.
Left side					1 (reference)	0.048		
Right side					0.71 (0.50-0.997)			
Tumor invasion								
T1&T2	1 (reference)		1 (reference)		1 (reference)		1 (reference)	
T3	2.61 (0.95-7.16)	0.063	2.79 (0.99-7.91)	0.054	2.94 (1.08-8.02)	0.036	2.73 (0.996-7.50)	0.051
T4	5.83 (2.06-16.48)	0.001	7.51 (2.58-21.84)	<0.001	6.41 (2.27-18.06)	<0.001	8.02 (2.83-22.77)	<0.001
Haemangiosis							n.d.	n.d.
No	1 (reference)		1 (reference)		1 (reference)			
Yes	1.85 (1.17-2.92)	0.008	1.61 (0.97-2.67)	0.065	1.84 (1.20-2.83)	0.005		
Stage								
Stage II	1 (reference)		1 (reference)		1 (reference)		1 (reference)	
Stage III	2.05 (1.39-3.04)	<0.001	2.55 (1.62-4.01)	<0.001	2.088 (1.439-3.029)	<0.001	2.86 (1.87-4.37)	<0.001
Adjuvant chemotherapy	n.d.	n.d.			n.d.	n.d.		
No			1 (reference)				1 (reference)	
Yes			0.51 (0.33-0.80)	0.003			0.51 (0.34-0.78)	0.002

SNP, Single-nucleotide polymorphism; TTR, time to recurrence; OS, overall survival; HR, hazard ratio; CI, confidence interval; n.d., not done.

with the homozygous A/A variant (hazard ratio (HR)=0.70, 95% confidence interval (CI)=0.50-0.95, p=0.036) in the dominant model. Patients carrying the homozygous A/A variant in SNP rs187115 had a probability of 5-year recurrence of 40.0% (SD=4.1), compared to patients carrying at least one G allele 29.3% (SD=2.9). The association with TTR remained significant in multivariable analysis after adjusting for age, tumor location, tumor invasion, count of lymph nodes resected, lymphatic, vascular and neural invasion, as well as tumor stage (HR=0.67, 95% CI=0.48-0.94, p=0.019) (Table II, Figure 1). The other gene variants tested, CD44 rs7116432, DPP4 rs2268889, CD44 rs353639, DPP4 rs3788979, DPP4 rs7608798, CD44 rs13347 and CD133 rs2240688, did not show a statistically significant association with TTR in the univariable analyses.

One hundred and sixty-eight out of 599 patients (28.0%) died during the observation period with a 5-year survival probability of 71.7% (SD=2.1). The SNP CD44 rs13347 showed a statistically significant association with OS in univariable analysis. Patients carrying at least one T allele in rs13347 had a significantly reduced risk of death compared to patients with the homozygous C/C variant (HR=0.62, 95% CI=0.42-0.93, p=0.019). Patients carrying the homozygous C/C in rs13347 had a 5-year survival probablity of 67.6% (SD=3.4) compared to patients carrying one C allele or the homozygous T/T 76.7% (SD=3.8). This result remained significant in multivariable analysis, including age, tumor location, tumor invasion depth, count of resected lymph nodes, lymphangiosis, hemangiosis, neural invasion, tumor stage and application of adjuvant chemotherapy (HR=0.61, 95% CI=0.41-0.92, p=0.019) (Table II, Figure 2).

The other tested gene variants CD44 rs187116, CD44 rs7116432, DPP4 rs2268889, CD44 rs353639, DPP4 rs3788979, DPP4 rs7608798, CD44 rs187115 and CD133 rs2240688 did not show a statistically significant association with OS in the univariable analyses.

We further investigated a possible association between clinicopathologic parameters and the two prognostically relevant SNPs (rs13347 and rs187115). However, neither rs187115 nor rs13347 was associated with stage of disease or any other clinicopathological parameter (Table III). Because

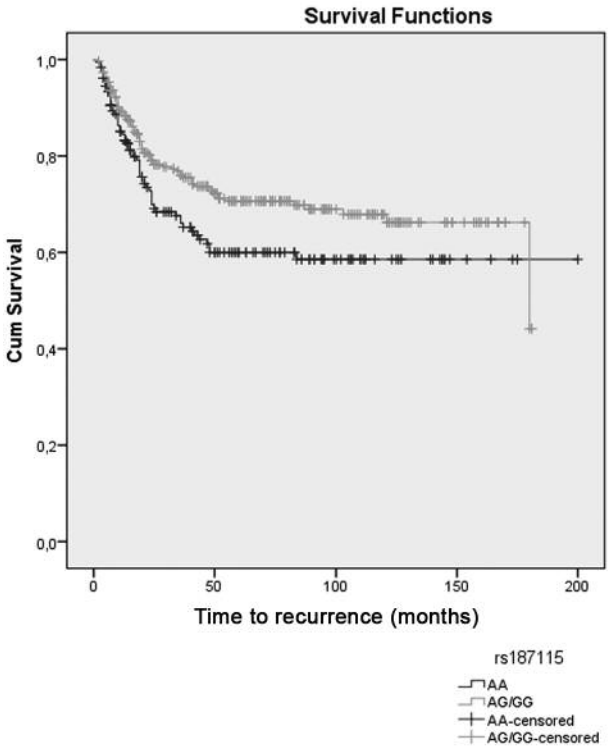

Figure 1. *Association of SNP rs187115 and TTR (n=599). SNP, Single-nucleotide polymorphism; TTR, time to recurrence.*

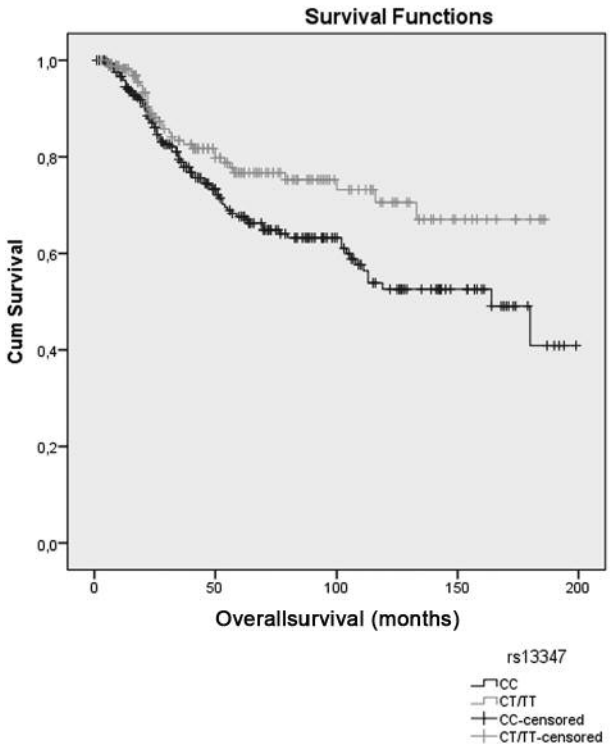

Figure 2. *Association of SNP rs13347 and OS (n=599). SNP, Single-nucleotide polymorphism; OS, overall survival.*

all other investigated SNPs showed no associations with TTR and OS, an association study for clinicopathological parameters was not performed for these SNPs because of missing clinical relevance.

Discussion

The *CD44* gene is located on the short arm of chromosome 11, is 50 kb long and consists of 20 exons, 12 of which are involved in splicing mechanisms. (21) It is a transmembrane glycoprotein that fulfills several functions in cell biology as adhesion, signaling and division by binding several ligands, including hyaluronic acid (HA). Cell-cell communication, as well as signal transduction, is influenced by CD44. Moreover, it interacts with the epidermal growth factor receptor (EGFR) (22) and shows activity in the regulation of the inflammatory response (23, 24). A strong CD44 expression in neoplastic crypts and advanced adenomas is indicative of its role in tumorigenesis of the gastrointestinal tract. Additionally, it has been identified as a CSC marker in colon cancer (14, 25). However, CD44 is not exclusive of colon cancer, since its isoforms are heterogeneously expressed in breast cancer and correlate with breast cancer subtypes (23). Nonetheless, the exact effect of altered CD44 expression remains unclear and has to be elucidated more clearly, particularly because given data suggest an important role in human cancers.

It is now widely accepted that DNA sequence variations can lead to altered gene function and/or activity, including transcription, translation or splicing, which could explain inter-individual differences in patients' clinical outcome (26). Among the nine SNPs investigated in the present study, rs13347 and rs187115 showed a prognostic value in stage II and III colorectal cancer patients. Recently, the role of SNP rs13347, as well as rs187115, was investigated in non-small cell lung cancer (NSCLC) patients. Interestingly, the group of Liu *et al*. could not discover any association between the SNP rs13347 and NSCLC risk, whereas rs187115 was significantly associated with survival. Allele G carriers had a significantly higher rate of bone metastasis ($p<0.001$) and a more advanced tumor stage ($p=0.001$) compared to carriers of the allele A. The survival rates for patients with AA genotype were significantly higher than for patients with the AG+GG genotypes ($p<0.001$) (27). This is contrary to our findings in CRC patients, as we were unable to show an association between rs187115 and altered survival rates. On the other hand, we could demonstrate an association for

Table III. *Baseline patients' characteristics and their association with SNP rs13347 and SNP rs187115 in univariable analysis.*

Parameter	SNP 13347					SNP 187115				
	CC	CT/TT	Miss	OR (95% CI)	*p*-Value	AA	AG/GG	Miss	OR (95% CI)	*p*-Value
Gender										
Male	131	107	92	1 (reference)	0.104	108	161	61	1 (reference)	0.211
Female	120	71	78	0.72 (0.49-1.07)		75	141	53	1.26 (0.87-1.83)	
Age										
in years				1.00 (0.98-1.02)	0.887				1.00 (0.98-1.01)	0.772
Tumor location										
Right	89	71	64	1 (reference)	0.350	68	111	45	1 (reference)	0.929
Left	162	107	106	0.83 (0.56-1.23)		115	191	69	1.02 (0.70-1.49)	
Lymph node operated										
≤12	35	20	29	1 (reference)	0.409	22	43	19	1 (reference)	0.488
>12	216	158	141	1.28 (0.71-2.30)		161	259	95	0.82 (0.48-1.43)	
Tumor invasion										
T1 & T2	18	10	7	1 (reference)	0.697	13	20	2	1 (reference)	0.846
T3	179	133	122	1.34 (0.60-2.99)		132	225	77	1.11 (0.53-2.30)	
T4	54	35	41	1.17 (0.48-2.82)		38	57	35	0.98 (0.43-2.19)	
Lymph node involvement										
N0	100	77	60	1 (reference)	0.627	74	123	40	1 (reference)	0.437
N1	94	67	68	0.93 (0.60-1.43)		63	117	49	1.12 (0.73-1.70)	
N2	57	34	42	0.78 (0.46-1.30)		46	62	25	0.81 (0.50-1.31)	
Tumor grade										
G1 & G2	181	121	116	1 (reference)	0.356	127	212	79	1 (reference)	0.852
G3	70	57	54	1.22 (0.80-1.85)		56	90	35	0.96 (0.65-1.44)	
Lymphovascular invasion										
No	181	130	124	1 (reference)	0.833	134	219	82	1 (reference)	0.865
Yes	70	48	46	0.96 (0.62-1.47)		49	83	32	1.04 (0.69-1.57)	
Vascular invasion										
No	224	160	155	1 (reference)	0.830	162	272	105	1 (reference)	0.592
Yes	27	18	15	0.93 (0.50-1.75)		21	30	9	0.85 (0.47-1.54)	
Perineural invasion										
No	243	174	167	1 (reference)	0.563	176	295	113	1 (reference)	0.341
Yes	8	4	3	0.70 (0.21-2.36)		7	7	1	0.60 (0.21-1.73)	
Stage										
II	100	75	60	1 (reference)	0.634	73	123	39	1 (reference)	0.966
III	151	103	110	0.91 (0.62-1.34)		110	179	75	0.97 (0.66-1.40)	
Adjuvant chemotherapy										
No	91	59	58	1 (reference)	0.506	66	108	34	1 (reference)	0.946
Yes	160	119	112	1.15 (0.77-1.72)		117	194	80	1.01 (0.69-1.49)	

SNP, Single-nucleotide polymorphism; TTR, time to recurrence; OS, overall survival; OR, odds ratio; CI, confidence interval; Miss, missing.

rs187115 regarding TTR, which was significantly higher in patients with the AG or GG genotype compared to patients with the homozygous AA genotype.

Stracquadanio *et al*. investigated the role of CD44 SNPrs187115 in pancreatic adenocarcinoma patients and could demonstrate an up to 2.38-fold increased risk for tumor-related death for mutated genotypes AG/GG in their cohort, which is also contrary to our findings in colorectal cancer (28). However, both Stracquadanio and Liu included metastasized patients in their cohorts, different to our study. Wu *et al*. showed that the C to T base change of rs13347 disrupts the binding site for hsa-mir-509-3p and increases the transcriptional activity of the *CD44* gene. They showed that patients with rs13347 CT and TT genotypes harbored significantly higher *CD44* mRNA levels compared to carriers of the rs13347CC genotypes. In their study, the variant genotypes CT and TT increased an individual's susceptibility to CRC by 1.6-fold, compared with rs13347 CC. Interestingly, they also discovered a more profound risk effect of this polymorphism in tumor stages III and IV (29). In our study, however, the homozygous genotype rs13347 CC was associated with a reduced survival rate compared to patients harboring the CT or TT genotype. An explanation for this diversity may be related to the fact that we did not include

stage IV patients in our cohort. Therefore, the SNP rs13347 may actually be of different prognostic value in adjuvant *versus* metastatic situations analogous to the role of microsatellite instability status in distinct colorectal cancer stages (30).

A limitation of our study is its retrospective design; therefore, a selection bias cannot be fully excluded. Another limitation is that frequencies of polymorphisms vary between different ethnicities.

In conclusion, we discovered rs187115 as being an independent prognostic biomarker regarding TTR, as well as rs13347 concerning OS, in stage II and III colorectal cancer patients. Nevertheless, prospective trials are needed to validate these promising genetic biomarkers in colon cancer patients.

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