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Genetic Variants in the Regulatory T cell–Related Pathway and Colorectal Cancer Prognosis

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ABSTRACT

Background: High numbers of lymphocytes in tumor tissue, including T regulatory cells (Treg), have been associated with better colorectal cancer survival. Tregs, a subset of $CD4^+$ T lymphocytes, are mediators of immunosuppression in cancer, and therefore variants in genes related to Treg differentiation and function could be associated with colorectal cancer prognosis.

Methods: In a prospective German cohort of 3,593 colorectal cancer patients, we assessed the association of 771 single-nucleotide polymorphisms (SNP) in 58 Treg-related genes with overall and colorectal cancer–specific survival using Cox regression models. Effect modification by microsatellite instability (MSI) status was also investigated because tumors with MSI show greater lymphocytic infiltration and have been associated with better prognosis. Replication of significant results was attempted in 2,047 colorectal cancer patients of the International Survival Analysis in Colorectal Cancer Consortium (ISACC).

Results: A significant association of the *TGFBR3* SNP rs7524066 with more favorable colorectal cancer–specific survival [hazard ratio (HR) per minor allele: 0.83; 95% confidence interval (CI), 0.74–0.94; *P* value: 0.0033] was replicated in ISACC (HR: 0.82; 95% CI, 0.68–0.98; *P* value: 0.03). Suggestive evidence for association was found with two *IL7* SNPs, rs16906568 and rs7845577. Thirteen SNPs with differential associations with overall survival according to MSI in the discovery analysis were not confirmed.

Conclusions: Common genetic variation in the Treg pathway implicating genes such as *TGFBR3* and *IL7* was shown to be associated with prognosis of colorectal cancer patients.

Impact: The implicated genes warrant further investigation.

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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Introduction

Colorectal cancer is the third most common cancer in the world (1). Implementation of population screening and the availability of new treatments have led to a decline in colorectal cancer–specific mortality and an increase in the 5-year survival (2–5). Colorectal cancer prognosis is very heterogeneous and dependent on several factors. The main prognostic factors that have been identified include TNM stage, tumor grade, presence of metastases (especially in the liver), baseline alkaline phosphatase levels, baseline C–reactive protein and albumin levels (Glasgow prognostic score), as well as neutrophil lymphocyte ratio and preoperative carcinoembryonic antigen (CEA) levels (6–10). In addition, the tumor microenvironment has been increasingly recognized to influence the prognosis of cancer including colorectal cancer (11). T regulatory cells (Treg), a subset of $CD4^+$ T lymphocytes expressing the transcription factor FOXP3, are heterogeneous cell types, which play a central role in the maintenance of self-tolerance and immune homeostasis by suppressing the activation, proliferation, and function of numerous immune cells (12, 13). In tumor tissue, Tregs are able to suppress antitumor immune response and contribute to the development of an immunosuppressive tumor microenvironment. The presence of high numbers of Tregs has a negative prognostic effect on many cancer types such as breast cancer, melanoma, or cervical cancer (14). In contrast, for colorectal cancer patients, the presence of a high number of tumor-infiltrating lymphocytes, including Tregs, in the tumor microenvironment has been associated with a more favorable survival (14–22). Tumors with microsatellite instability (MSI) are frequently characterized by inflammatory lymphocytic infiltration and tend to be associated with a better survival than non–MSI-high colorectal cancers (23–25). This may, in part, be due to more effective immune responses involving Tregs (26, 27).

Genetic variation in inflammatory genes could play a role in the survival of patients after colorectal cancer diagnosis (28). To gain further insight into the biological mechanisms underlying Treg pathway and survival after colorectal cancer, we investigated common, inherited single-nucleotide polymorphisms (SNP) affecting genes involved in the regulation of Treg functions.

No studies have so far investigated a possible influence of genetic variants in Treg-related genes on the prognosis of colorectal cancer patients. Therefore, our aim was to investigate the association between 771 germline variants in 58 Treg-related genes and the overall disease-specific survival of colorectal cancer patients, and to assess possible effect modification by MSI status.

Materials and Methods

The study sample consisted of colorectal cancer patients recruited into the ongoing population-based case–control study DACHS (**Da**rmkrebs: **Ch**ancen der Verhütung durch **S**creening) conducted in the Rhine-Neckar Odenwald region in southwestern Germany (29, 30). Cases diagnosed between January 2003 and December 2013 were included if they were older than 30 years of age (with no upper limit), were able to communicate in German, were able to participate in a personal interview of around 1 hour and were a resident of the Rhine-Neckar Odenwald region. Only histologically confirmed cases who were diagnosed with their first primary colorectal cancer (ICD-10:C18-C20) were included. All patients gave their written informed consent. The study was approved by the relevant ethical committees of the University of Heidelberg and the State Medical Boards of Baden-Württemberg and Rhineland-Palatinate, Germany, and was conducted in agreement with the Declaration of Helsinki.

At baseline, trained interviewers collected information on the patient's demographics, anthropometric indices, medical history including reproductive history, and lifestyle factors. A blood sample was requested at baseline, and for a minority of patients who refused to provide blood, a mouthwash sample was collected instead (0.9% of participants). After five years of follow-up, information on treatment and disease course was collected from the treating physician. Vital status was collected from the population registries, and cause of death was verified by death certificates from health authorities.

Genotype data

The Flexigene kit was used to extract DNA from EDTA blood and mouthwash samples of the DACHS patients, and quantification of the DNA was performed using Q6 Quanti-iT picoGreen dsDNA reagent and kit (Invitrogen/Life Technologies).

Genotyping was performed in collaboration with the Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO); details have been previously described (31). DACHS samples were genotyped using the whole-genome Illumina CytoSNP assay (Illumina) for patients recruited in 2003–2006, the Illumina HumanOmniExpress BeadChip Kit for patients recruited in 2007–2010, and the Illumina HumanOmniExpress BeadChip Kit or the Illumina Infinium OncoArray-500K BeadChip for those recruited in 2011–2013. For quality control, genotyped variants were excluded based on call rate (<98%), lack of Hardy–Weinberg equilibrium in controls (HWE; $P < 1 \times 10^{-4}$), and low minor allele frequency (MAF <0.05) as described elsewhere (31–34). Samples were imputed using as reference panel the cosmopolitan haplotypes from phase I of the 1,000 Genome Project (for patients recruited between 2003 and 2010) or the Haplotype Reference Consortium (for patients between 2011 and 2013; ref. 35) using the University of Michigan Imputation Server (36). Before imputation, Shapeit2 was used to phase the GWAS data (37).

SNP selection

Through extensive literature research, the most important genes in the Treg pathway were selected. Tagging SNPs were selected to represent genetic variation across the genes. SNPs in these genes as well as SNPs in the flanking regions (e.g., ±10 kb) were considered, after which Haploview 4.2 (Broad Institute) was used for the selection of tagging SNPs, with a pairwise tagging approach based on reference data from the HapMap project [Utah resident with Northern and Western Europe ancestry (CEU population), Phase II/Release 24]. In total, 771 SNPs in 58 genes were selected for this analysis (see Supplementary Table S1).

MSI data

Formalin-fixed paraffin-embedded (FFPE) tumor samples were used to determine MSI status. FFPE samples were collected from the different pathology departments at the cooperating hospitals and were stored at the tissue bank at the National Center for Tumor Diseases (NCT) in Heidelberg. The area with the highest tumor cell concentration was identified microscopically, and this section was then isolated using the DNeasy kit from Qiagen. A mononucleotide marker panel including BAT25, BAT26, and CAT25 was used to determine MSI status. Tumors showing amplifications in two or more markers were classified as MSI-high (38). These markers have a sensitivity of 98.2% and a specificity of 100% to differentiate MSI-high from non–MSI-high tumors (38, 39).

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Data analysis

Cox regression models were used to test the individual SNP associations with overall survival and colorectal cancer–specific survival. Hazard ratios (HR) and 95% confidence intervals (CI) were calculated for each SNP. Survival time was calculated from date of diagnosis until date of death by any cause, death by colorectal cancer, or date of last contact. Median follow-up time was calculated using the reverse Kaplan–Meier method (40). Age, sex, and TNM stage were included in the model as relevant prognostic factors. Additional covariates were determined using backward elimination of a set of variables including grade (1, 2 vs. 3, 4), family history of colorectal cancer in first-degree relatives (no vs. yes), smoking, body mass index (18.5–25, 25–30, 30+ kg/m^2), alcohol intake (0 and quartiles in subjects with alcohol intake >0 g/day), and physical activity (0 and quartiles). The variables body mass index (at diagnosis) and alcohol intake were retained in the final model as they were significantly associated with overall survival. Heterogeneity in the associations of the Treg gene polymorphisms with overall survival according to MSI status was assessed statistically using interaction terms between MSI status (high, non-high) and the polymorphisms and was evaluated using the likelihood ratio test. The SNP association according to MSI status was also estimated in subgroup analysis. The proportional hazards assumption was tested according to Grambsch and Therneau (41). The statistical analysis was carried out using SAS version 9.3 (SAS Institute) and R version 3.1.0 (www.r-project.org).

Replication set

For SNPs that showed a significant association with survival or an interaction with MSI ($P < 0.01$), replication was performed using studies participating in the International Survival Analysis in Colorectal Cancer Consortium (ISACC), a consortium aimed at investigating demographic, environmental, and genetic risk factors in association with colorectal cancer survival (42). For replication of the SNP associations with overall survival and colorectal cancer–specific survival, 1,821 colorectal cancer patients from six studies were included: Diet And Lifestyle Study (DALS), Health Professionals Follow-up Study (HPFS), Nurses' Health Study (NHS), Colon Cancer Family Registry (CCFR), Cancer Prevention Study II (CPS II), and the Melbourne Collaborative Cohort Study (MCCS). All studies were genotyped on Illumina GWAS platforms and imputed to the Haplotype Reference Consortium panel using the University of Michigan Imputation Server (35). Prior to imputation, Shapeit2 was used to phase the GWAS data (37). Details of genotyping and quality control (QC) for studies included in the validation are described elsewhere (32–34, 43). Patients included in ISACC studies are of European ancestry.

For replication of results on effect modification according to MSI status, 1,554 colorectal cancer patients from five studies (DALS, HPFS, NHS, CPS II, and MCCS) with available data on MSI were included. For DALS, MSI status was determined using 12 markers, a panel of 10 tetranucleotide repeats and the two mononucleotide repeats (BAT-26 and TGFBR2; ref. 44). For HPFS and NHS, MSI status was assessed based on the same 10 tetranucleotide repeats (45). For MCCS, MSI status was determined using a 10-loci panel in tumor DNA and matched normal tissue DNA (BAT25, BAT26, BAT40, MYCL, D5S346, D17S250, ACTC, D18S55, D10S197, and BAT34C4; ref. 46). For CPS II, determination of MSI status was based on the Bethesda Consensus Panel (47). Classification was based on ≥5 interpretable markers (unless all four markers were unstable, in which case the tumor was classified as MSI-high). For these studies, MSI-high tumors were defined when ≥30% of the markers showed instability.

Analyses were performed by pooling the samples from all studies and adjusting for study. We used the same statistical methods as for the discovery phase. To account for the overrepresentation of patients with a positive family history of colorectal cancer in the DALS Minnesota samples, we included family history as an additional covariate in the MSI interaction analysis given the correlation between positive family history and MSI-high tumors. Missing data on environmental factors were imputed using single imputation.

Functional annotation

To add functional information to significant variants, we used the NCI's "LDlink" web tool (https://ldlink.nci.nih.gov) to find all variants in linkage disequilibrium (LD; $R^2 \geq 0.4$ in phase III 1000 Genomes "EUR" population) with the significant variant. Subsequently, we used the variant effect predictor (VEP) tool of the Ensembl webpage (https://uswest.ensembl.org/info/docs/tools/vep/index.html) to show annotations (48).

Data availability

Genotyping data of the GECCO studies are available at the database of Genotypes and Phenotypes (dbGaP) for download at the accession number phs001078.v1.p1.

Results

Table 1 shows the characteristics of the DACHS study participants. The majority of patients were aged between 60 and 80 years at diagnosis with a median of 69 years, and 60.5% were male. Over 60% of the patients were diagnosed with TNM stage II or III disease, and 60.2% had a tumor in the colon. The median follow-up time was 63.0 months and, in total, 1,100 patients died during study follow-up. See the flow diagram in **Fig. 1** for inclusion of patients in the discovery stage. In the DACHS study, 10.7% of patients with data on MSI status were MSI-high and 89.3% were non–MSI-high.

Three SNPs showed an association with overall survival (nominal $P < 0.01$) in the single SNP analysis (**Table 2**; see Supplementary Table S2 for results of all SNPs). The minor alleles of two genetic variants were associated with an increased risk of dying, rs2290065 (*CCR7*) with a HR of 1.31 per allele (95% CI, 1.07–1.61) and rs10815237 (*CD274*) with a HR of 1.13 per minor allele (95% CI, 1.04–1.23). The minor allele of rs2421826 (*CD44*) was associated with lower overall survival (HR: 0.89; 95% CI, 0.82–0.97).

For colorectal cancer–specific survival, nine SNPs were associated at $P < 0.01$. Here, the minor alleles of two SNPs in each of the genes *IL7* (rs7845577 and rs16906568; LD r2 = 0.35) and *TGFBR2* (rs1495578 and rs17623772; LD r2 = 0.8) as well as rs10815237 in gene *CD274* were associated with poorer colorectal cancer–specific survival. The latter SNP rs10815237 was also associated with overall survival. The minor alleles of the other four SNPs, two in *TGFBR3* (rs7524066 and rs17571088; LD r2 = 0.36) and one each in *TGFB3* (rs4252328) and *CD44* (rs2421826), were associated with better colorectal cancer–specific survival (**Table 2**; see Supplementary Table S3 for all SNPs).

Differential associations by MSI status

The results of the effect modifications by MSI status (211 MSI-high tumors; 1,754 non–MSI-high) are shown for all SNPs in Supplementary Table S4. In the replication sample (ISACC), 18.3% of samples were MSI-high and 81.7% non–MSI-high. Thirteen SNPs showed statistically significant interaction (nominal $P < 0.01$) with MSI status (**Table 3**). Three of the SNPs lie in gene

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Table 1. Basic characteristics for 3,593 colorectal cancer DACHS patients with complete follow-up information and for 1,965 colorectal cancer patients according to MSI status.

N (number of events)	All 3,593 (1,100) *n* (%)	MSI-high 211 (50) *n* (%)	Non–MSI-high 1,754 (590) *n* (%)
Sex			
Female	1,420 (39.5)	110 (52.1)	712 (40.6)
Male	2,173 (60.5)	101 (47.9)	1,042 (59.4)
Age, years			
<60	744 (20.7)	36 (17.1)	333 (19.0)
60–<70	1,123 (31.3)	59 (28.0)	661 (31.4)
70–<80	1,189 (33.1)	61 (28.9)	607 (34.6)
≥80	537 (14.9)	55 (26.1)	263 (15.0)
Median (Interquartile range)	69 (61–76)	71 (64–80)	69 (62–76)
TNM stage			
1	820 (22.8)	30 (14.2)	325 (18.7)
2	1,088 (30.3)	113 (53.6)	554 (31.6)
3	1,188 (32.9)	62 (28.4)	612 (34.9)
4	502 (14.0)	6 (2.8)	260 (14.8)
Site			
Colon	2,164 (60.2)	200 (94.8)	1,036 (59.1)
Rectum	1,429 (39.8)	11 (5.2)	718 (40.9)
CRC-specific death			
No	2,811 (78.2)	191 (90.5)	1,334 (76.1)
Yes	718 (20.0)	19 (9.0)	404 (23.0)
Missing	64 (1.8)	1 (0.5)	16 (0.9)
Recurrence			
No	2,576 (71.7)	185 (87.7)	1,206 (68.8)
Yes	983 (27.4)	25 (11.8)	542 (30.9)
Missing	34 (0.9)	1 (0.5)	6 (0.3)
BMI category			
Normal weight	1,390 (38.7)	65 (30.8)	704 (40.1)
Overweight	1,527 (42.5)	91 (43.1)	728 (41.5)
Obese	676 (18.8)	55 (26.1)	322 (18.4)
Current alcohol intake (g/day)			
No alcohol	1,093 (30.4)	74 (35.1)	524 (29.9)
0.1–6.1	705 (19.6)	52 (124.6)	331 (18.9)
6.1–15.6	624 (17.4)	34 (16.1)	303 (17.3)
15.6–32.6	607 (16.9)	31 (14.7)	306 (17.4)
≥32.6	564 (15.7)	20 (9.5)	290 (16.5)
Family history of CRC			
No	3,086 (85.9)	174 (82.5)	1,505 (85.8)
Yes	507 (14.1)	37 (17.5)	249 (14.2)

Abbreviations: BMI, body mass index; CRC, colorectal cancer.

CD4 (rs7957426, rs10774451, and rs10849524), the minor alleles of two of which were associated with decreased survival in MSI-high tumors (rs7957426 and rs10774451) and of one SNP rs10849524 was associated with increased survival in MSI-high tumors. These SNPs were not associated with survival in non–MSI-high tumors. Five of the SNPs that showed significant heterogeneity were annotated to gene *HLA-DRA*, of which the minor alleles of four SNPs were also associated with decreased survival (rs3129848, rs17496549, rs3135392, and rs9268644) in MSI-high tumors. None of these five SNPs were associated with survival in non–MSI-high tumors. Two SNPs in *IL15RA* (rs2228059 and rs1998521) also showed associations with overall survival, although in different directions for the minor alleles, and only in MSI-high tumors but not in non–MSI-high tumors. The remaining SNPs, rs2069772 (*IL2*), rs11165376 (*TGFBR3*), and rs7135373 (*IFNG*), were also found associated with decreased survival solely in MSI-high tumors.

Replication analysis

The characteristics of the participants from the ISACC studies included in the replication analysis are shown in Supplementary Table S5. None of the SNPs associated with overall survival in the DACHS discovery set showed significant association with overall survival in the ISACC replication sample (see **Table 2**). One of the nine associations with improved colorectal cancer–specific survival in DACHS, for rs7524066 (*TGFBR3*), was confirmed in the replication analysis, with a similar magnitude of association (HR: 0.82; 95% CI, 0.68–0.98). Two more SNPs (rs16906568 and rs7845577) in gene *IL7*, associated with poorer survival, were replicated with similar effect sizes and borderline significance ($P = 0.05$ and 0.07, respectively). Although not reaching statistical significance, most SNPs showed the same direction of association in the replication sample except for rs4252328 (*TGFB3*). None of the SNPs that showed differential association by MSI status in DACHS were confirmed in ISACC.

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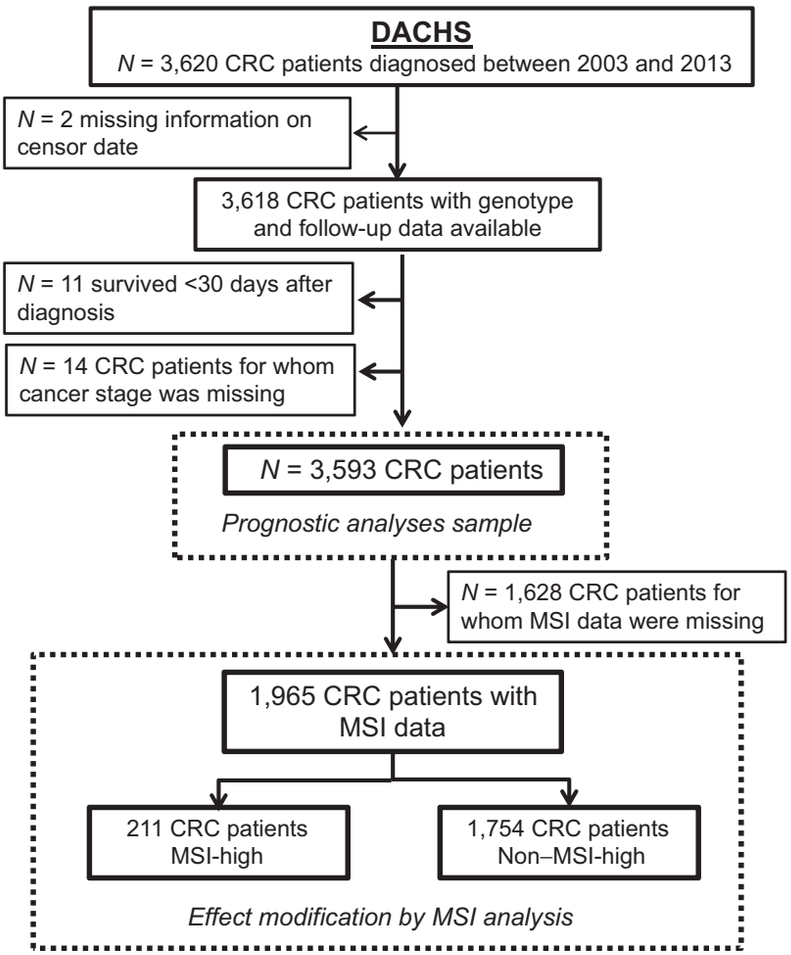

Figure 1.
Flow diagram on patients' participation in the discovery stage. CRC, colorectal cancer.

Table 2. SNPs in Treg-related genes showing associations with overall survival in 3,593 colorectal cancer DACHS patients at nominal $P < 0.01$ and replication in independent 2,047 colorectal cancer patients.

		DACHS		ISACC	
SNP	Gene	HR per minor allele (95% CI)	*P* value	HR per minor allele (95% CI)	*P* value
Overall survival					
rs10815237	*CD274*	1.13 (1.04–1.23)	0.0064	1.06 (0.94–1.19)	0.38
rs2421826	*CD44*	0.89 (0.82–0.97)	0.0090	0.98 (0.87–1.10)	0.71
rs2290065	*CCR7*	1.31 (1.07–1.61)	0.0095	0.92 (0.72–1.19)	0.87
CRC-specific survival					
rs10815237	*CD274*	1.19 (1.07–1.32)	0.0018	1.14 (0.97–1.35)	0.16
rs7524066	*TGFBR3*	0.83 (0.74–0.94)	0.0033	0.82 (0.68–0.98)	0.03
rs17571088	*TGFBR3*	0.82 (0.71–0.94)	0.0050	0.95 (0.78–1.17)	0.65
rs1495578	*TGFBR2*	1.17 (1.05–1.30)	0.0046	1.09 (0.94–1.27)	0.25
rs17623772	*TGFBR2*	1.16 (1.04–1.29)	0.0081	1.07 (0.91–1.25)	0.41
rs4252328	*TGFB3*	0.84 (0.73–0.95)	0.0070	1.06 (0.90–1.27)	0.48
rs16906568	*IL7*	1.18 (1.05–1.33)	0.0050	1.20 (1.00–1.43)	0.05
rs7845577	*IL7*	1.22 (1.05–1.42)	0.0084	1.23 (0.98–1.55)	0.07
rs2421826	*CD44*	0.87 (0.78–0.97)	0.0098	0.92 (0.79–1.08)	0.31

Note: HRs, CIs, and *P* values are estimated from Cox proportional hazards regression analysis. Models were adjusted by age, sex, TNM stage, BMI at diagnosis, and current alcohol intake.
Abbreviations: BMI, body mass index; CRC, colorectal cancer.

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Table 3. SNPs in Treg-related genes showing differential association with overall survival according to MSI status in 1,965 colorectal cancer patients (at nominal *P* value for interaction <0.01) and replication in independent 1,307 colorectal cancer patients.

		DACHS					ISACC				
		MSI-high (211 CRC patients)		Non–MSI-high (1,754 CRC patients)			MSI-high (239 CRC patients)		Non–MSI-high (1,068 CRC patients)		
SNP	Gene	HR per minor allele (95% CI)	*P* value	HR per minor allele (95% CI)	*P* value	*P*-value interaction	HR per minor allele (95% CI)	*P* value	HR per minor allele (95% CI)	*P* value	*P*-value interaction
Overall survival											
rs7957426	*CD4*	2.13 (1.39–3.26)	0.0005	1.00 (0.89–1.13)	0.9754	0.0035	1.28 (0.81–2.02)	0.2888	0.94 (0.82–1.07)	0.3493	0.3445
rs3129848	*CD4*	2.00 (1.28–3.12)	0.0024	0.97 (0.86–1.09)	0.5737	0.0045	1.58 (0.99–2.53)	0.0573	0.92 (0.81–1.05)	0.2209	0.3800
rs10774451	*CD4*	0.51 (0.32–0.80)	0.0031	1.02 (0.90–1.14)	0.7973	0.0052	1.59 (1.00–2.54)	0.0501	0.91 (0.81–1.04)	0.1711	0.3195
rs17496549	*HLA-DRA*	2.09 (1.20–3.64)	0.0088	1.16 (0.97–1.39)	0.0995	0.0052	0.49 (0.25–0.96)	0.0389	1.34 (1.11–1.62)	0.0026	0.2190
rs10849524	*HLA-DRA*	1.75 (1.10–2.78)	0.0179	0.97 (0.85–1.10)	0.6514	0.0041	0.50 (0.30–0.84)	0.0080	1.15 (1.00–1.32)	0.0513	0.3972
rs3135392	*HLA-DRA*	1.50 (1.00–2.26)	0.0512	0.93 (0.82–1.05)	0.2387	0.0067	0.59 (0.37–0.94)	0.0269	0.99 (0.87–1.14)	0.9220	0.1468
rs2069772	*HLA-DRA*	1.51 (0.99–2.31)	0.0554	0.96 (0.85–1.07)	0.4497	0.0097	2.30 (1.38–3.81)	0.0013	0.82 (0.72–0.93)	0.0027	0.8181
rs6911419	*HLA-DRA*	0.72 (0.48–1.09)	0.1165	1.10 (0.98–1.23)	0.0939	0.0075	2.28 (1.38–3.75)	0.0012	0.90 (0.79–1.03)	0.1388	0.5347
rs2228059	*IL15RA*	1.80 (1.15–2.82)	0.0107	0.92 (0.82–1.03)	0.1455	0.0075	0.98 (0.64–1.49)	0.9113	0.96 (0.84–1.10)	0.5549	0.8178
rs1998521	*IL15RA*	0.60 (0.38–0.94)	0.0267	1.03 (0.92–1.16)	0.6011	0.0076	0.86 (0.55–1.34)	0.5042	1.00 (0.87–1.14)	0.9774	0.6695
rs11165376	*TGFBR3*	0.54 (0.31–0.93)	0.0275	0.98 (0.86–1.12)	0.8021	0.0082	1.13 (0.69–1.85)	0.6343	0.92 (0.81–1.06)	0.2581	0.5994
rs7135373	*IFNG*	0.47 (0.27–0.82)	0.0079	0.99 (0.87–1.12)	0.8357	0.0089	1.07 (0.65–1.77)	0.7889	0.92 (0.80–1.05)	0.2149	0.7597
rs9268644	*IL2*	0.56 (0.33–0.95)	0.0326	0.98 (0.86–1.11)	0.7510	0.0075	1.51 (0.95–2.39)	0.0830	0.95 (0.82–1.09)	0.4507	0.6975

Note: HRs, CIs, and corresponding *P* values are estimated from Cox proportional hazards regression analysis. Interaction *P* values calculated using likelihood ratio tests comparing the model with and without interaction term.

Abbreviation: CRC, colorectal cancer.

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Functional genomic annotation

The SNP rs7524066 (*TGFBR3*), for which the association with disease-specific survival was replicated, was further investigated to add functional information. We found 33 SNPs to be in LD with the investigated variant ($R^2 > 0.4$; Supplementary Table S6). We further assessed the function of these variants using the VEP tool of the Ensembl webpage (Supplementary Table S7). We found that 15 of the LD SNPs are located in regulatory regions of the gene. Two of the SNPs are located in transcription factor binding sites (Supplementary Table S7).

Discussion

We investigated the association of 771 Treg-related genetic variants with overall and colorectal cancer–specific survival in a large cohort of colorectal cancer patients and performed replication of top findings in an independent cohort of colorectal cancer patients from the ISACC consortium. Although none of the SNPs associated with overall survival were confirmed in the independent data set, one of nine SNPs associated with colorectal cancer–specific survival in the discovery data set, rs7524066 (*TGFBR3*), was confirmed in the independent replication data set. The minor allele was similarly associated with better colorectal cancer–specific survival in the discovery sample (HR: 0.83; 95% CI, 0.74–0.94) and the replication sample (HR: 0.82; 95% CI, 0.68–0.98).

Based on previous observations of differential T-cell infiltration of colorectal tumors according to MSI status (26, 49, 50), we also evaluated SNPs' association with overall survival by MSI status. We found 13 SNPs in six different genes that showed interactions with MSI in the discovery set ($P < 0.01$) but were not able to replicate any of these interactions.

The SNP rs7524066, for which the association was replicated in the primary analysis of colorectal cancer–specific survival, is annotated to gene *TGFBR3* (transforming growth factor β type III receptor), encoding one of the transforming growth factor β (TGFβ) receptors, which is also known as betaglycan (51). TGFβ is an important growth factor for normal development and homeostasis of all cells in the human body and can have both tumor-suppressor and tumor-promoting functions depending on context (52, 53). In contrast to the other two TGFβ receptors, *TGFBR1* and *TGFBR2, TGFBR3* does not have kinase activity (53). Still, it is not only a coreceptor but seems to act as a tumor suppressor for several cancer types (54, 55). *TGFBR3* appears to suppress WNT/CTNNB1 (β-catenin) signaling (56), which is linked to colorectal cancer development and progression. Our *in silico* functional analyses indicated that several SNPs in LD with rs7524066 lie in regulatory regions of the gene *TGFBR3* and therefore might modify gene regulation. These mechanisms support the plausibility of *TGFBR3* being associated with colorectal cancer–specific survival.

Furthermore, the association with worse colorectal cancer–specific survival of the minor allele of two SNPs (rs16906568 and rs7845577) related to gene *IL7* (LD r2 = between the SNPs = 0.35) is of interest. *IL7* is a cytokine that is important for B- and T-cell development. Expression of IL7 was higher in colorectal cancer patients compared with controls and was associated with metastatic disease (57). Therefore, *IL7* variants could have a role in survival after colorectal cancer diagnosis.

The *CD274* variant rs10815237 was associated with both overall and colorectal cancer–specific survival and showed fairly similar magnitude of association particularly for colorectal cancer–specific survival in the replication sample albeit nonsignificant. Tumor CD274 [programmed cell death ligand 1 (PD-L1)] expression has been associated inversely with Treg density in colorectal cancer (58). Tumor CD274 expression may modify prognostic association of aspirin (59). These data suggest that CD274 (PD-L1) may modify colorectal cancer behavior depending on other factors in the tumor immune microenvironment. It would be of interest to examine the interaction of the *CD274* variant and Treg density (or aspirin use) in future prognostic studies.

One SNP (rs2421826) in the *CD44* gene was also found associated with improved both overall and colorectal cancer–specific survival in the discovery analysis but was not statistically significantly associated in the replication set. *CD44* is a multistructural and multifunctional cell-surface adhesion molecule that is highly expressed in many cancers and involved in physiologic processes. Through interaction with extracellular matrix ligands, it promotes the migration and invasion processes involved in metastases. Functionally active *CD44* is associated with enhanced suppressor activity of Treg (60). Expression of stem-like factors including *CD44* has been associated with metastatic disease and poorer prognosis in colorectal cancer (61) Two SNPs in gene *TGFBR2* were associated with worse colorectal cancer–specific survival, but were not replicated. One of the roles of *TGFBR2* is Treg suppression (62), and its inactivation has been associated with the development of colorectal cancer (63). These mechanisms make it plausible that genetic variants in *TGFBR2* could be associated with colorectal cancer–specific survival.

We were not able to confirm any of the results stratified by MSI status, which could be partly due to the difference in the panels used for MSI characterization. The inability to confirm many of the SNPs associated with overall or colorectal cancer–specific survival in the discovery sample could also be in part due to the limited power because the replication sample was smaller than the discovery sample. New association findings are generally biased upward in discovery data sets so that larger study samples are required for replication. Measurement of Treg/FOXP3 cell expression within the tumor may improve future studies investigating Treg-related SNP associations with colorectal cancer survival.

Research into Tregs remains challenging as the definition of Tregs has changed over the past decade. The interplay with other T helper cells, expression of surface markers, as well as expression of cytokines influencing functionality of T-cell subtypes add to the complexity of this research field. Several studies have shown that different factors, including activated state of the immune cells, their location in the cellular matrix, and ratio of different immune cells, can influence their involvement in tumor progression and subsequent colorectal cancer survival (21, 64). Two distinct subpopulations of Tregs were recently identified and shown to have differential impact on colorectal cancer prognosis (65). Genetic variation, which can be robustly measured, could help to provide further evidence for the prognostic impact of Tregs on colorectal cancer prognosis.

This study is one of the first studies investigating genetic variations in the Treg pathway with respect to colorectal cancer survival. Replication was attempted in an independent sample of colorectal cancer patients. A large amount of genotype data were available, which enabled the comprehensive investigation of the Treg pathways. The quality control measures all showed that the genotype data were of high quality. There were no opportunities to perform functional analyses for the SNPs that were implicated through these analyses.

Although no strong associations were found, there is suggestive evidence based on these analyses, particularly for the TGFβ

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receptors and the biological functions of the implicated genes, to support further investigations of the aforementioned SNPs and genes with respect to colorectal cancer prognosis in large study samples.

Disclosure of Potential Conflicts of Interest

A.T. Chan reports personal fees from Bayer Pharma AG, Pfizer Inc., and Boehringer Ingelheim outside the submitted work. M. Gala has equity in New Amsterdam Genomics, Inc. This firm has not provided any funding for the research involved or had any role in study design. The firm provides clinical sequencing to medical providers and patients. S. Ogino reports grants from National Institutes of Health (R35 CA197735) during the conduct of the study. C.M. Ulrich reports being Cancer Center Director. Dr. Ulrich oversees all research activities, including some funded by pharmaceutical industry. Dr. Ulrich has personally not received any funds from for-profit institutions or corporations in the past 5 years. F. Macrae reports other from Rhythym Biosciences (funding support for testing diagnostic for colorectal cancer) outside the submitted work, as well as receiving aspirin from Bayer for the Australian CaPP3 noninferiority dose-finding trial in Lynch syndrome. The trial is supported by the Victorian Cancer Agency. R.L. Milne reports grants from National Health and Medical Research Council during the conduct of the study. H. Brenner reports grants from the German Federal Ministry of Education and Research during the conduct of the study. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

S. Neumeyer: Conceptualization, methodology, writing–original draft, project administration, writing–review and editing. **X. Hua:** Data curation, software, formal analysis, writing–review and editing. **P. Seibold:** Validation, writing–review and editing. **L. Jansen:** Data curation. **A. Benner:** Validation and methodology. **B. Burwinkel:** Validation and methodology. **N. Halama:** Data curation and validation. **S.I. Berndt:** Conceptualization, resources, funding acquisition, writing–review and editing. **A.I. Phipps:** Funding acquisition, writing–review and editing. **L.C. Sakoda:** Resources, supervision, writing–review and editing. **R.E. Schoen:** Resources, funding acquisition, writing–review and editing. **M.L. Slattery:** Resources, funding acquisition, writing–review and editing. **A.T. Chan:** Resources, funding acquisition, writing–review and editing. **M. Gala:** Resources, funding acquisition, writing–review and editing. **A.D. Joshi:** Resources, funding acquisition, writing–review and editing. **S. Ogino:** Resources, funding acquisition, writing–review and editing. **M. Song:** Resources, funding acquisition, writing–review and editing. **E. Herpel:** Data curation and validation. **H. Bläker:** Resources, data curation, and validation. **M. Kloor:** Resources, validation, and investigation. **D. Scherer:** Investigation and methodology. **A. Ulrich:** Investigation and methodology. **C.M. Ulrich:** Resources, funding acquisition, writing–review and editing. **A.K. Win:** Resources, funding acquisition, writing–review and editing. **J.C. Figueiredo:** Resources, funding acquisition, writing–review and editing. **J.L. Hopper:** Resources, funding acquisition, writing–review and editing. **F. Macrae:** Resources, funding acquisition, writing–review and editing. **R.L. Milne:** Resources and funding acquisition. **G.G. Giles:** Resources and funding acquisition. **D.D. Buchanan:** Resources, funding acquisition, and investigation. **U. Peters:** Resources, data curation, funding acquisition, investigation, writing–review and editing. **M. Hoffmeister:** Conceptualization, resources, funding acquisition, validation, investigation, writing–review and editing. **H. Brenner:** Resources, data curation, funding acquisition, writing–review and editing. **P.A. Newcomb:** Resources, funding acquisition, investigation, methodology, writing–review and editing. **J. Chang-Claude:** Conceptualization, resources, supervision, funding acquisition, investigation, methodology, writing–original draft, writing–review and editing.

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References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394–424.
2. Benning TM, Dellaert BGC, Dirksen CD, Severens JL. Preferences for potential innovations in non-invasive colorectal cancer screening: a labeled discrete choice experiment for a Dutch screening campaign. Acta Oncol 2014;53:898–908.
3. Blom J, Kilpeläinen S, Hultcrantz R, Törnberg S. Five-year experience of organized colorectal cancer screening in a Swedish population–increased compliance with age, female gender, and subsequent screening round. J Med Screen 2014;21:144–50.
4. Brenner H, Stock C, Hoffmeister M. Effect of screening sigmoidoscopy and screening colonoscopy on colorectal cancer incidence and mortality: systematic review and meta-analysis of randomised controlled trials and observational studies. BMJ 2014;348:g2467.
5. Price TJ, Segelov E, Burge M, Haller DG, Ackland SP, Tebbutt NC, et al. Current opinion on optimal treatment for colorectal cancer. Expert Rev Anticancer Ther 2013;13:597–611.

6. Braun MS, Richman SD, Quirke P, Daly C, Adlard JW, Elliott F, et al. Predictive biomarkers of chemotherapy efficacy in colorectal cancer: results from the UK MRC FOCUS trial. J Clin Oncol 2008;26:2690–8.
7. McMillan DC. The systemic inflammation-based Glasgow Prognostic Score: a decade of experience in patients with cancer. Cancer Treat Rev 2013;39:534–40.
8. Tsai PL, Su WJ, Leung WH, Lai C-T, Liu C-K. Neutrophil-lymphocyte ratio and CEA level as prognostic and predictive factors in colorectal cancer: a systematic review and meta-analysis. J Cancer Res Ther 2016;12:582–9.
9. Templeton AJ, McNamara MG, Šeruga B, Vera-Badillo FE, Aneja P, Ocaña A, et al. Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: a systematic review and meta-analysis. J Natl Cancer Inst 2014;106:dju124.
10. Ishizuka M, Nagata H, Takagi K, Iwasaki Y, Kubota K. Inflammation-based prognostic system predicts postoperative survival of colorectal cancer patients with a normal preoperative serum level of carcinoembryonic antigen. Ann Surg Oncol 2012;19:3422–31.
11. Quail DF, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. Nat Med 2013;19:1423–37.
12. Sakaguchi S, Miyara M, Costantino CM, Hafler DA. FOXP3+ regulatory T cells in the human immune system. Nat Rev Immunol 2010;10:490–500.
13. Savage PA, Malchow S, Leventhal DS. Basic principles of tumor-associated regulatory T cell biology. Trends Immunol 2013;34:33–40.
14. Shang B, Liu Y, Jiang S-J, Liu Yi. Prognostic value of tumor-infiltrating FoxP3+ regulatory T cells in cancers: a systematic review and meta-analysis. Sci Rep 2015; 5:15179.
15. Chew A, Salama P, Robbshaw A, Klopcic B, Zeps N, Platell C, et al. SPARC, FOXP3, CD8 and CD45 correlation with disease recurrence and long-term disease-free survival in colorectal cancer. PLoS One 2011;6:e22047.
16. Kim M, Grimmig T, Grimm M, Lazariotou M, Meier E, Rosenwald A, et al. Expression of Foxp3 in colorectal cancer but not in Treg cells correlates with disease progression in patients with colorectal cancer. PLoS One 2013;8:e53630.
17. Correale P, Rotundo MS, Botta C, Del Vecchio MT, Tassone P, Tagliaferri P. Tumor infiltration by chemokine receptor 7 (CCR7)(+) T-lymphocytes is a favorable prognostic factor in metastatic colorectal cancer. Oncoimmunology 2012;1:531–2.
18. Berntsson J, Svensson MC, Leandersson K, Nodin B, Micke P, Larsson AH, et al. The clinical impact of tumour-infiltrating lymphocytes in colorectal cancer differs by anatomical subsite: a cohort study. Int J Cancer 2017;141:1654–66.
19. Waniczek D, Lorenc Z, Śnietura M, Wesecki M, Kopec A, Muc-Wierzgoń M. Tumor-associated macrophages and regulatory T cells infiltration and the clinical outcome in colorectal cancer. Arch Immunol Ther Exp 2017;65: 445–54.
20. Hanke T, Melling N, Simon R, Sauter G, Bokemeyer C, Lebok P, et al. High intratumoral FOXP3(+) T regulatory cell (Tregs) density is an independent good prognosticator in nodal negative colorectal cancer. Int J Clin Exp Pathol 2015;8: 8227–35.
21. Zhuo C, Xu Ye, Ying M, Li Q, Huang L, Li D, et al. FOXP3+ Tregs: heterogeneous phenotypes and conflicting impacts on survival outcomes in patients with colorectal cancer. Immunol Res 2015;61:338–47.
22. Chen J, Chen Z. The effect of immune microenvironment on the progression and prognosis of colorectal cancer. Med Oncol 2014;31:82.
23. Buckowitz A, Knaebel HP, Benner A, Bläker H, Gebert J, Kienle P, et al. Microsatellite instability in colorectal cancer is associated with local lymphocyte infiltration and low frequency of distant metastases. Br J Cancer 2005;92: 1746–53.
24. Frey DM, Droeser RA, Viehl CT, Zlobec I, Lugli A, Zingg U, et al. High frequency of tumor-infiltrating FOXP3(+) regulatory T cells predicts improved survival in mismatch repair-proficient colorectal cancer patients. Int J Cancer 2010;126: 2635–43.
25. Prall F, Duhrkop T, Weirich V, Ostwald C, Lenz P, Nizze H, et al. Prognostic role of CD8+ tumor-infiltrating lymphocytes in stage III colorectal cancer with and without microsatellite instability. Hum Pathol 2004;35:808–16.
26. Bauer K, Nelius N, Reuschenbach M, Koch M, Weitz J, Steinert G, et al. T cell responses against microsatellite instability-induced frameshift peptides and influence of regulatory T cells in colorectal cancer. Cancer Immunol Immunother 2013;62:27–37.
27. Kim K-J, Lee KS, Cho HJ, Kim YH, Yang HK, Kim WHo, et al. Prognostic implications of tumor-infiltrating FoxP3+ regulatory T cells and CD8+ cytotoxic T cells in microsatellite-unstable gastric cancers. Hum Pathol 2014; 45:285–93.
28. Bondurant KL, Lundgreen A, Herrick JS, Kadlubar S, Wolff RK, Slattery ML. Interleukin genes and associations with colon and rectal cancer risk and overall survival. Int J Cancer 2013;132:905–15.
29. Lilla C, Verla-Tebit E, Risch A, Jäger B, Hoffmeister M, Brenner H, et al. Effect of NAT1 and NAT2 genetic polymorphisms on colorectal cancer risk associated with exposure to tobacco smoke and meat consumption. Cancer Epidemiol Biomarkers Prev 2006;15:99–107.
30. Verla-Tebit E, Lilla C, Hoffmeister M, Brenner H, Chang-Claude J. Cigarette smoking and colorectal cancer risk in Germany: a population-based case-control study. Int J Cancer 2006;119:630–5.
31. Peters U, Jiao S, Schumacher FR, Hutter CM, Aragaki AK, Baron JA, et al. Identification of genetic susceptibility loci for colorectal tumors in a genome-wide meta-analysis. Gastroenterology 2013;144:799–807.
32. Schumacher FR, Schmit SL, Jiao S, Edlund CK, Wang H, Zhang B, et al. Genome-wide association study of colorectal cancer identifies six new susceptibility loci. Nat Commun 2015;6:7138.
33. Schmit SL, Edlund CK, Schumacher FR, Gong J, Harrison TA, Huyghe JR, et al. Novel common genetic susceptibility loci for colorectal cancer. J Natl Cancer Inst 2019;111:146–57.
34. Huyghe JR, Bien SA, Harrison TA, Kang HM, Chen S, Schmit SL, et al. Discovery of common and rare genetic risk variants for colorectal cancer. Nat Genet 2019; 51:76–87.
35. McCarthy S, Das S, Kretzschmar W, Delaneau O, Wood AR, Teumer A, et al. A reference panel of 64,976 haplotypes for genotype imputation. Nat Genet 2016; 48:1279–83.
36. Das S, Forer L, Schönherr S, Sidore C, Locke AE, Kwong A, et al. Next-generation genotype imputation service and methods. Nat Genet 2016;48:1284–7.
37. Delaneau O, Howie B, Cox AJ, Zagury J-F, Marchini J. Haplotype estimation using sequencing reads. Am J Hum Genet 2013;93:687–96.
38. Findeisen P, Kloor M, Merx S, Sutter C, Woerner SM, Dostmann N, et al. T25 repeat in the 3' untranslated region of the CASP2 gene: a sensitive and specific marker for microsatellite instability in colorectal cancer. Cancer Res 2005;65: 8072–8.
39. Carr PR, Jansen L, Bienert S, Roth W, Herpel E, Kloor M, et al. Associations of red and processed meat intake with major molecular pathological features of colorectal cancer. Eur J Epidemiol 2017;32:409–18.
40. Schemper M, Smith TL. A note on quantifying follow-up in studies of failure time. Control Clin Trials 1996;17:343–6.
41. Grambsch PM, Therneau TM. Proportional hazards tests and diagnostics based on weighted residuals. Biometrika 1994;81:515–26.
42. Chong DQ, Banbury BL, Phipps AI, Hua X, Kocarnik J, Peters U, et al. Association of family history and survival in patients with colorectal cancer: a pooled analysis of eight epidemiologic studies. Cancer Med 2018;7:2192–9.
43. Peters U, Jiao S, Schumacher FR, Hutter CM, Aragaki AK, Baron JA, et al. Identification of genetic susceptibility loci for colorectal tumors in a genome-wide meta-analysis. Gastroenterology 2013;144:799–807.
44. Slattery ML, Anderson K, Curtin K, Ma KN, Schaffer D, Samowitz W. Dietary intake and microsatellite instability in colon tumors. Int J Cancer 2001;93:601–7.
45. Lee JE, Baba Y, Ng K, Giovannucci E, Fuchs CS, Ogino S, et al. Statin use and colorectal cancer risk according to molecular subtypes in two large prospective cohort studies. Cancer Prev Res 2011;4:1808–15.
46. Buchanan DD, Clendenning M, Rosty C, Eriksen SV, Walsh MD, Walters RJ, et al. Tumor testing to identify Lynch syndrome in two Australian colorectal cancer cohorts. J Gastroenterol Hepatol 2017;32:427–38.
47. Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, et al. A National Cancer Institute workshop on microsatellite instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res 1998;58:5248–57.
48. McLaren W, Pritchard B, Rios D, Chen Y, Flicek P, Cunningham F. Deriving the consequences of genomic variants with the Ensembl API and SNP effect predictor. Bioinformatics 2010;26:2069–70.
49. Deschoolmeester V, Baay M, Van ME, Weyler J, Vermeulen P, Lardon F, et al. Tumor infiltrating lymphocytes: an intriguing player in the survival of colorectal cancer patients. BMC Immunol 2010;11:19.
50. Lee SY, Miyai K, Han HS, Hwang D-Y, Seong MK, Chung H, et al. Microsatellite instability, EMAST, and morphology associations with T cell infiltration in colorectal neoplasia. Dig Dis Sci 2012;57:72–8.

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51. Gatza CE, Holtzhausen A, Kirkbride KC, Morton A, Gatza ML, Datto MB, et al. Type III TGF-beta receptor enhances colon cancer cell migration and anchorage-independent growth. Neoplasia 2011;13:758–70.
52. Massague J. TGFbeta in cancer. Cell 2008;134:215–30.
53. Vander Ark A, Cao J, Li X. TGF-beta receptors: in and beyond TGF-beta signaling. Cell Signal 2018;52:112–20.
54. Dong M, How T, Kirkbride KC, Gordon KJ, Lee JD, Hempel N, et al. The type III TGF-beta receptor suppresses breast cancer progression. J Clin Invest 2007;117:206–17.
55. Turley RS, Finger EC, Hempel N, How T, Fields TA, Blobe GC. The type III transforming growth factor-beta receptor as a novel tumor suppressor gene in prostate cancer. Cancer Res 2007;67:1090–8.
56. Jenkins LM, Singh P, Varadaraj A, Lee NY, Shah S, Flores HV, et al. Altering the proteoglycan state of transforming growth factor beta type III receptor (TbetaRIII)/betaglycan modulates canonical Wnt/beta-catenin signaling. J Biol Chem 2016;291:25716–28.
57. Krzystek-Korpacka M, Zawadzki M, Neubauer K, Bednarz-Misa I, Górska S, Wiśniewski J, et al. Elevated systemic interleukin-7 in patients with colorectal cancer and individuals at high risk of cancer: association with lymph node involvement and tumor location in the right colon. Cancer Immunol Immunother 2017;66:171–9.
58. Masugi Y, Nishihara R, Yang J, Mima K, da Silva A, Shi Y, et al. Tumour CD274 (PD-L1) expression and T cells in colorectal cancer. Gut 2017;66:1463–73.
59. Hamada T, Cao Y, Qian ZR, Masugi Y, Nowak JA, Yang J, et al. Aspirin use and colorectal cancer survival according to tumor CD274 (programmed cell death 1 ligand 1) expression status. J Clin Oncol 2017;35:1836–44.
60. Firan M, Dhillon S, Estess P, Siegelman MH. Suppressor activity and potency among regulatory T cells is discriminated by functionally active CD44. Blood 2006;107:619–27.
61. Fang C, Fan C, Wang C, Huang Q, Meng W, Yu Y, et al. Prognostic value of CD133+ CD54+ CD44+ circulating tumor cells in colorectal cancer with liver metastasis. Cancer Med 2017;6:2850–7.
62. Lan Q, Zhou X, Fan H, Chen M, Wang J, Ryffel B, et al. Polyclonal CD4+Foxp3+ Treg cells induce TGFbeta-dependent tolerogenic dendritic cells that suppress the murine lupus-like syndrome. J Mol Cell Biol 2012;4:409–19.
63. Muñoz NM, Upton M, Rojas A, Washington MK, Lin Li, Chytil A, et al. Transforming growth factor beta receptor type II inactivation induces the malignant transformation of intestinal neoplasms initiated by Apc mutation. Cancer Res 2006;66:9837–44.
64. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pagès C, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science 2006;313:1960–4.
65. Saito T, Nishikawa H, Wada H, Nagano Y, Sugiyama D, Atarashi K, et al. Two FOXP3(+)CD4(+) T cell subpopulations distinctly control the prognosis of colorectal cancers. Nat Med 2016;22:679–84.

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