The Pharmacogenomics Journal (2018) 18:556–564
https://doi.org/10.1038/s41397-017-0009-x

ARTICLE

Genetic variants in the VEGF pathway as prognostic factors in stages II and III colon cancer

Pau Riera[1,2] · Anna C. Virgili[3,4] · Juliana Salazar[1,5] · Ana Sebio[3] · María Tobeña[3] · Ivana Sullivan[3] · David Páez[3]

Received: 9 June 2017 / Revised: 19 September 2017 / Accepted: 6 November 2017 / Published online: 27 December 2017

Abstract

The role of vascular endothelial growth factor (VEGF) gene polymorphisms in the prognosis of colon cancer prognosis remains unclear. We evaluated the influence of 28 single-nucleotide polymorphisms in 12 genes in the VEGF pathway on the prognosis of 347 patients with stage II–III colon cancer. We found that rs9513070 (*VEGFR1*) and rs1137282 (*KRAS*) were associated with overall survival in stage II colon cancer patients ($p = 0.025$ and $p = 0.001$, respectively). When primary tumor location was considered, rs9513070 was also associated with relapse-free and overall survival ($p = 0.033$ and $p = 0.031$, respectively) in left colon cancer patients. Additionally, rs35251833 in the *ITGAV* gene correlated with relapse-free survival ($p = 0.032$). This study provides evidence that germline polymorphisms in *VEGFR1*, *KRAS* and *ITGAV* genes are associated with prognosis in stages II–III colon cancer patients. As stage and tumor location are correlated with prognosis, future genetic studies should stratify colon cancer patients according to these parameters.

Introduction

The survival of colon cancer (CC) has improved in recent decades, however this disease remains the third cause of cancer death worldwide [1]. Patients with stages II and III CC undergo a complete surgical resection with curative intent. However, the risk of tumor recurrence is considerable, especially in patients with stage III and high-risk stage II disease. In these stages, adjuvant chemotherapy following surgery is recommended.

Several biomarkers have been correlated with prognosis of CC. Microsatellite instability, for example, implies a favorable outcome in stage II patients. Other putative biomarkers include *BRAF*, *KRAS PIK3CA* mutations and CDX2 expression, but few studies have described the role of molecules such as VEGF and interleukins in the likelihood of recurrence in stage II–III colon cancer [2–5].

The vascular endothelial growth factor (VEGF) pathway plays a key role in tumor-induced angiogenesis, which promotes the growth and progression of solid tumors [6, 7]. Activation of this pathway may confer a worse prognosis in stages II–III colon cancer as it can promote the switch from dormant tumor cells not removed by surgery to proliferative cells [8]. Multiple proteins are involved in the VEGF pathway, particularly VEGFA, a major mediator of angiogenesis whose synthesis is stimulated under hypoxic conditions by HIFα. Several receptors (VEGFR1/FLT1, VEGFR2/KDR, VEGFR3 and FGFR), neuropilins (NRP1) and integrins (ITGAV), among other proteins are involved in the process. The binding of circulating VEGFA to its main receptors, VEGFR1 and VEGFR2, promotes cell migration, survival and proliferation by triggering a protein cascade that involves PRKCE, GRB2, RAS and MAP kinases [9–11].

Heritable functional variation in genes involved in the angiogenesis process may impact on the angiogenic switch, tumor progression and, hence, on clinical cancer outcomes. In the present work, we analyzed 28 genetic variants in a panel of 12 VEGF-dependent genes (*VEGFA, VEGFR1/FLT1, VEGFR2/KDR, GRB2, ITGAV, KISS1, KRAS, PRKCE, HIF1α, MAP2K4, MAP2K6, MAPK11*). The goal

Pau Riera and Anna C. Virgili contributed equally to this study.

✉ Juliana Salazar
jsalazar@santpau.cat

1 Genetics Department, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain

2 Universitat de Barcelona (UB), Barcelona, Spain

3 Medical Oncology Department, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain

4 Universitat Autònoma de Barcelona (UAB), Barcelona, Spain

5 CIBERER, U-705 Barcelona, Spain

Table 1 Selected polymorphisms in VEGF-related genes

SNP	Gene symbol	Alleles	MAF (%)	Rationale for genotyping (ref.)
rs7219	*GRB2*	T>C	26	LCL eQTLs [12]
rs11549465	*HIF1α*	C>T	10	mCRC survival [13]
rs35251833	*ITGAV*	G>A	31	LCL eQTLs [12]
rs71745629	*KISS1*	T>*	22	mCRC survival [14]
rs61764370	*KRAS*	T>G	10	Let7 [15]
rs10842513	*KRAS*	C>T	9	LCL eQTLs [12]
rs12813551	*KRAS*	T>C	40	Stage I–III NSCLC RFS [16]
rs1137282	*KRAS*	A>G	22	Stage I–III NSCLC RFS [17]
rs3826392	*MAP2K4*	T>G	25	mCRC survival [14]
rs11656130	*MAP2K6*	T>G	45	mCRC PFS [14]
rs2716191	*MAP2K6*	T>C	48	mCRC PFS [14]
rs2076139	*MAPK11*	C>T	26	LCL eQTLs [12]
rs4953299	*PRKCE*	T>C	24	LCL eQTLs [12]
rs833061	*VEGFA*	T>C	50	mCRC PFS [18]
rs1570360	*VEGFA*	G>A	32	mCRC PFS [18]
rs2010963	*VEGFA*	G>C	31	mCRC OS [19]
rs3025039	*VEGFA*	C>T	12	Stage III CRC RFS [5]
rs699947	*VEGFA*	C>A	50	mCRC OS [19]
rs3024997	*VEGFA*	G>A	31	LCL eQTLs [12]
rs9582036	*VEGFR1*	A>C	27	Stage I–III NSCLC RFS [16]
rs7993418	*VEGFR1*	A>G	20	Advanced NSCLC survival [20]
rs9513070	*VEGFR1*	A>G	41	mCRC survival [21]
rs7996030	*VEGFR1*	G>A	20	Stage I–III NSCLC RFS [16]
rs2305948	*VEGFR2*	C>T	9	mCRC PFS [14]
rs7667298	*VEGFR2*	C>T	45	mCRC survival [14]
rs1551641	*VEGFR2*	G>A	30	mCRC survival [14]
rs2071559	*VEGFR2*	A>G	49	mCRC survival [14]
rs1870377	*VEGFR2*	T>A	23	Functional evidence [22]

GRB2 growth factor receptor-bound protein 2, *HIF1α* hypoxia inducible factor 1, alpha subunit, *ITGAV* integrin, alpha V, *KISS1* KiSS-1 metastasis-suppressor, *KRAS* kirsten rat sarcoma viral oncogene homolog, *MAP2K4* mitogen-activated protein kinase kinase 4, *MAP2K6* mitogen-activated protein kinase kinase 6, *MAPK11* mitogen-activated protein kinase 11, *PRKCE* protein kinase C, epsilon, *VEGFA* vascular endothelial growth factor A, *VEGFR1* or *FLT1* fms-related tyrosine kinase 1, *VEGFR2* or *KDR* kinase insert domain receptor, *eQTLs* quantitative trait loci expressions, *LCL* lymphoblastoid cell lines, *MAF* minor allele frequency (1000 Genomes Project, European population; accession date: 25/04/17), *mCRC* metastatic colorectal cancer, *NSCLC* non-small cell lung cancer, *RFS* relapse-free survival, *PFS* progression-free survival

of this study was to evaluate the association between the selected genetic variants and the clinical outcome in a cohort of 347 stages II–III CC patients.

Materials and methods

Patient population

Three-hundred and forty-seven patients with stage II–III CC who underwent radical surgery between 2009 and 2014 at Hospital de la Santa Creu i Sant Pau (HSCSP, Barcelona, Spain) were consecutively included in the study. Patients with rectal tumor were excluded. Patient data including tumor localization, histological tumor grade, lymph node sampling and vascular or perineural invasion were collected retrospectively through chart review. Patients were treated with fluoropyrimidine-based adjuvant chemotherapy at clinician discretion. Blood samples from 345 patients were available for the genetic analyses. The study was approved by the Institutional Ethics Committee at HSCSP and all the study participants gave informed consent for the analysis of molecular correlates.

Genetic studies

We analyzed 28 single-nucleotide polymorphisms (SNPs) in 12 candidate genes involved in the VEGF pathway. The selected SNPs were (i) SNPs associated with lymphoblastoid cell line (LCL) mRNA expression of VEGF pathway genes [12] and (ii) functional variants associated with cancer survival according to studies reported in the literature [5, 13–22]. The SNPs that were Tag SNPs had an r^2 greater than 0.8. All SNPs had a minor allele frequency (MAF) >5% in the Caucasian population. Table 1 shows detailed information about the SNPs.

Genomic DNA was automatically extracted from peripheral whole-blood samples (Autopure, Qiagen, Hilden, Germany). SNP genotyping was performed by means of real-time PCR using TaqMan® SNP genotyping assays (Applied Biosystems, Foster City, CA, USA) and 48.48 dynamic arrays on the BioMarkTM system (Fluidigm, San Francisco, CA, USA). Patients' characteristics and clinical outcome were unknown to the investigator conducting the genetic analyses. SNP allele frequencies were comparable to those reported in the 1000 Genomes project. Genotyping was successful in atleast 99% of cases for each SNP analyzed. The quantity and/or the quality of the extracted DNA were the most common causes of failure.

Statistical analyses

The endpoints of the study were recurrence-free survival (RFS) and overall survival (OS). RFS was calculated from the date of surgery until the date of first recurrence. RFS was censored at the last follow-up or the time of death if the patient remained tumor recurrence-free at that time. OS was defined as the time from the surgery date until death from any cause or last follow-up. We also analyzed survival after relapse (SaR), which was defined as the time from the relapse date until death from any cause or last follow-up. All patients were included in the colon cancer surveillance program of HSCSP, providing history and undergoing physical examination and CEA determination every 3 months for 2 years and every 6 months at years 3–5 after surgery, colonoscopy at year 1 and thereafter every 3–5 years, and computed tomographic scans of chest and abdomen every year. The associations between polymorphisms and clinicopathological features were evaluated using Fisher's exact test. The associations of polymorphisms with RFS and OS were analyzed using Kaplan–Meier curves and a log-rank test. Three different models of inheritance were considered to evaluate associations with outcome variables—additive, dominant and recessive. To identify markers associated with the outcomes independently from clinical data, each SNP was fitted to a Cox model in which the statistically significant clinicopathological variables in the univariate analyses were included as covariates. For each SNP, Hardy–Weinberg equilibrium was assessed using an exact test. All statistical tests were performed at 95% significance. All analyses were performed using SPSS (version 19.0, IBM).

Results

We studied a total of 347 patients. Two-hundred and five were diagnosed as stage II and 142 were classified as stage III CC. Following total surgical resection of the tumor, 47 patients received adjuvant fluorouracil plus leucovorin (FL) or oral fluoropyrimidine capecitabine, and 162 patients were treated with oxaliplatin added to FL (FOLFOX) or capecitabine (XELOX). The remaining 138 patients did not receive adjuvant therapy.

Table 2 shows the baseline clinical characteristics. The median follow-up was 46.3 months (range, 9.3–91.5 months). Seventy-one patients (20.5%) relapsed during the follow-up. Thirty-four of the 71 corresponded to 16.6% of the stage II patients, while the remaining 37 patients represented 26.1% of the stage III group.

The stage II and stage III probability of 3-year recurrence-free survival was 0.876 ± 0.023 and 0.800 ± 0.034, respectively. The stage II and stage III probability of 5-year overall survival was 0.860 ± 0.025 and 0.766 ± 0.036, respectively.

Stage classification was significantly associated with RFS ($p = 0.028$) and OS ($p = 0.001$). Tumor grade was significantly associated with OS ($p = 0.032$). Localization of the tumor and administration of adjuvant therapy were associated with OS ($p = 0.05$). Perineural invasion and the

Table 2 Baseline patient characteristics

	n	%
Sex		
Male	193	55.6
Female	154	44.4
Age		
<75	212	61.1
≥75	135	38.9
T stage		
T1 and T2	17	4.9
T3	216	62.2
T4	114	32.9
Grade		
Low	287	82.7
High	48	13.8
Missing	12	3.5
N stage		
N0	205	59.1
N1	96	27.7
N2	46	13.3
Stage		
II	205	59.1
III	142	40.9
N of resected lymph nodes		
<12	57	16.4
≥12	290	83.6
Vascular invasion		
Yes	65	18.7
No	210	60.5
Missing	72	20.7
Perineural invasion		
Yes	49	14.1
No	223	64.3
Missing	75	21.6
Tumor side		
Right	143	41.2
Left	204	58.8
Adjuvant treatment		
5-FU/LV or capecitabine	47	13.5
5-FU/LV/oxaliplatin or capecitabine/oxaliplatin	162	46.7
No adjuvant therapy	138	39.8

number of resected lymph nodes were also significantly associated with RFS ($p = 0.004$ and $p = 0.05$, respectively). Thus, these covariates were included in the multivariate model.

Genetic determinants and RFS

In the univariate analysis of the stage II and III patients, two genetic variants were significantly associated with RFS: rs61764370 in the *KRAS* gene ($p = 0.041$) and rs699947 in the *VEGFA* gene ($p = 0.035$). These results are detailed in Table 3. Non-significant genetic variants are presented in the Supplementary Table. Stage II patients showed a significant association between RFS and four genetic variants: rs35251833 in the *ITGAV* gene ($p = 0.018$), rs71745629 in the *KISS1* gene ($p = 0.018$), rs61764370 in the *KRAS* gene ($p = 0.046$) and rs4953299 in the *PRKCE* gene ($p = 0.033$) (Table 4). No association was found between any of the SNPs evaluated and RFS in stage III colon cancer patients. In the multivariate analysis, none of the variants remained significantly associated with RFS.

Genetic determinants and OS

In the univariate analysis of the stage II and III patients, three genetic variants were significantly associated with OS: rs11656130 (*MAP2K6*) ($p = 0.017$), rs9513070 (*VEGFR1*) ($p = 0.041$) and rs1551641 (*VEGFR2*) ($p = 0.049$). Table 3 shows these results. Polymorphisms not significantly associated with OS are detailed in the Supplementary Table. Stage II patients showed a significant association between OS and six genetic variants corresponding to four different genes: rs11549465 (*HIF1α*) ($p = 0.003$); rs1137282 (*KRAS*) ($p = 0.001$); rs833061, rs1570360 and rs699947 (*VEGFA*) ($p = 0.038$, $p = 0.009$ and $p = 0.035$, respectively) and rs9513070 (*VEGFR1*) ($p = 0.025$) (Table 4). No association was found between any of the SNPs evaluated and OS in stage III colon cancer patients.

Table 3 Germline polymorphisms in the VEGF pathway and univariate analysis for time to recurrence and overall survival in patients with stage II or III colon cancer

Polymorphism	*N*	Time to recurrence			Overall survival		
		Probability ± s.e. of 3-year free recurrence	Hazard ratio (95% CI)	*P*-value	Probability ± s.e. of 5-year survival	Hazard ratio (95% CI)	*P*-value
KRAS rs61764370							
T/T	282	0.793 ± 0.025	1 (reference)	**0.041**	0.844 ± 0.033	1 (reference)	0.349
T/G[a]	59	0.952 ± 0.027	0.45 (0.2, 0.9)		0.964 ± 0.025	0.61 (0.2, 1.7)	
G/G[a]	4						
MAP2K6 rs11656130							
T/T	99	0.857 ± 0.036	1 (reference)	0.215	0.894 ± 0.044	1 (reference)	**0.017**
T/G	171	0.822 ± 0.030	1.28 (0.7, 2.3)		0.896 ± 0.034	1.13 (0.4, 2.8)	
G/G	75	0.770 ± 0.049	1.78 (0.9, 3.4)		0.719 ± 0.102	2.90 (1.1, 7.3)	
VEGFA rs699947							
C/C	97	0.882 ± 0.034	1 (reference)	**0.035**	0.902 ± 0.043	1 (reference)	0.346
C/A[a]	164	0.798 ± 0.026	1.89 (1.0, 3.4)		0.849 ± 0.035	1.49 (0.6, 3.4)	
A/A[a]	84						
VEGFR1 rs9513070							
A/A	112	0.763 ± 0.041	1 (reference)	0.133	0.812 ± 0.054	1 (reference)	**0.041**
A/G[a]	165	0.849 ± 0.024	0.69 (0.4, 1.1)		0.891 ± 0.030	0.49 (0.2, 0.9)	
G/G[a]	67						
VEGFR2 rs1551641							
G/G	164	0.832 ± 0.030	1 (reference)	0.755	0.932 ± 0.026	1 (reference)	**0.049**
G/A[a]	161	0.812 ± 0.030	0.93 (0.6, 1.5)		0.796 ± 0.048	2.03 (0.9, 4.2)	
A/A[a]	20						

P-value was based on log-rank test in codominant

s.e. Greedwood standard error

[a]Dominant model

[b]Recessive model

The bold values indicate the *P*-values statistically significant ($P < 0.05$)

Table 4 Germline polymorphisms in the VEGF pathway associated with RFS/OS by tumor stage

Recurrence free survival

	Stage II only				Stage III only			
Polymorphism	*N*	Probability ± s.e. of 3-year free recurrence	Hazard ratio (95% CI)	*P*-value	*N*	Probability ± s.e. of 3-year free recurrence	Hazard ratio (95% CI)	*P*-value
ITGAV rs35251833								
G/G	98	0.822 ± 0.039	1 (reference)	**0.006**	58	0.789 ± 0.054	1 (reference)	0.841
G/A	89	0.932 ± 0.027	0.39 (0.2, 0.9)		71	0.737 ± 0.054	1.04 (0.5, 2.0)	
A/A	17	0.706 ± 0.111	1.94 (0.8, 4.8)		12	0.825 ± 0.113	0.68 (0.1, 2.9)	
A/A[b]	17	0.706 ± 0.111	2.77 (1.1, 6.7)	**0.018**	12	0.825 ± 0.113	0.66 (0.1, 2.8)	0.570
KISS1 rs71745629								
T/T	123	0.818 ± 0.035	1 (reference)	**0.018**	74	0.787 ± 0.049	1 (reference)	0.587
T/*[a]	77	0.923 ± 0.030	0.38 (0.2, 0.9)		61	0.746 ± 0.053	1.19 (0.6, 2.3)	
/[a]	4				6			
KRAS rs61764370								
T/T	160	0.828 ± 0.030	1 (reference)	**0.046**	122	0.746 ± 0.040	1 (reference)	0.589
T/G[a]	40	0.977 ± 0.023	0.32 (0.1, 1.0)		19	0.895 ± 0.070	0.75 (0.3, 2.1)	
G/G[a]	4				0			
PRKCE rs4953299								
T/T	122	0.865 ± 0.031	1 (reference)	0.098	86	0.775 ± 0.046	1 (reference)	0.788
T/C	72	0.874 ± 0.039	0.88 (0.4, 1.8)		45	0.740 ± 0.069	1.26 (0.6, 2.5)	
C/C	10	0.700 ± 0.145	2.82 (0.9, 8.3)		10	0.800 ± 0.126	0.94 (0.2, 4.0)	
C/C[b]	10	0.700 ± 0.145	2.96 (1.0, 8.4)	**0.033**	10	0.800 ± 0.126	0.86 (0.2, 3.6)	0.842

Overall survival

	Stage II only				Stage III only			
Polymorphism	*N*	Probability ± s.e. of 5-year survival	Hazard ratio (95% CI)	*P*-value	*N*	Probability ± s.e. of 5-year survival	Hazard ratio (95% CI)	*P*-value
HIF1α rs11549465								
C/C	161	0.919 ± 0.033	1 (reference)	**0.008**	115	0.765 ± 0.058	1 (reference)	0.498
C/T	40	0.974 ± 0.026	0.39 (0.05, 3.1)		24	0.767 ± 0.175	0.49 (0., 2.1)	
T/T	30	0.667 ± 0.272	10.87 (1.3, 91.3)		2	–	–	
T/T[b]	30	0.667 ± 0.272	12.4 (1.5, 103.2)	**0.003**	2	–	–	0.53
KRAS rs1137282								
A/A	121	0.922 ± 0.034	1 (reference)	**0.002**	89	0.803 ± 0.061	1 (reference)	0.737
A/G	75	0.950 ± 0.049	0.50 (0.1, 2.4)		50	0.717 ± 0.103	1.18 (0.5, 2.8)	
G/G	8	0.750 ± 0.153	7.99 (1.5, 41.1)		2	–	–	
G/G[b]	8	0.750 ± 0.153	9.81 (1.9, 48.4)	**0.001**	2	–	–	0.494
VEGFA rs833061								
T/T	61	0.984 ± 0.016	1 (reference)	0.074	38	0.682 ± 0.139	1 (reference)	0.883
T/C	94	0.906 ± 0.048	3.28 (0.4, 28.1)		67	0.810 ± 0.080	0.77 (0.3, 2.2)	
C/C	49	0.902 ± 0.047	7.84 (0.9, 67.5)		36	0.773 ± 0.089	0.85 (0.3, 2.7)	
C/C[b]	49	0.902 ± 0.047	3.29 (0.9, 10.9)	**0.038**	36	0.773 ± 0.089	1.00 (0.4, 2.6)	0.991
VEGFA rs1570360								
G/G	93	0.954 ± 0.027	1 (reference)	**0.034**	64	0.768 ± 0.084	1 (reference)	0.250
G/A	93	0.900 ± 0.053	1.07 (0.3, 4.3)		65	0.735 ± 0.085	1.24 (0.5, 2.9)	
A/A	18	0.881 ± 0.079	5.14 (1.1, 23.2)		12	–	–	
A/A[b]	18	0.881 ± 0.079	4.96 (1.3, 18.9)	**0.009**	12	–	–	0.113

Table 4 (continued)

Overall survival

Polymorphism	Stage II only				Stage III only			
	N	Probability ± s.e. of 5-year survival	Hazard ratio (95% CI)	*P*-value	*N*	Probability ± s.e. of 5-year survival	Hazard ratio (95% CI)	*P*-value
VEGFA rs699947								
C/C	60	0.983 ± 0.017	1 (reference)	0.106	37	0.707 ± 0.142	1 (reference)	1.000
C/A	96	0.906 ± 0.048	3.94 (0.7, 20.4)		68	0.796 ± 0.080	1.00 (0.3, 3.3)	
A/A	48	0.899 ± 0.048	1.29 (0.2, 7.0)		36	0.773 ± 0.089	0.99 (0.3, 2.9)	
A/A[b]	48	0.899 ± 0.048	3.35 (1.0, 11.1)	**0.035**	36	0.773 ± 0.089	1.00 (0.4, 2.6)	0.991
VEGFR1 rs9513070								
A/A	61	0.907 ± 0.040	1 (reference)	**0.025**	51	0.669 ± 0.119	1 (reference)	0.362
A/G[a]	100	0.929 ± 0.037	0.27 (0.08, 0.9)		65	0.829 ± 0.053	0.67 (0.3, 1.6)	
G/G[a]	43				24			

P-value was based on log-rank test in codominant

s.e. Greedwood standard error

[a] Dominant model

[b] Recessive model

The bold values indicate the *P*-values statistically significant ($P < 0.05$)

In the multivariate analysis, two SNPs retained their significance: rs1137282 in the *KRAS* gene and rs9513070 in the *VEGFR1* gene. Stage II patients harboring a homozygous GG genotype in the *KRAS* SNP were significantly associated with decreased OS (HR = 10.82; 95% CI: 2.1–56.0; adjusted $p = 0.005$). In the same cohort of stage II CC patients, patients carrying at least one G allele of the SNP in the *VEGFR1* gene were significantly associated with an increased OS (HR = 0.268; 95% CI: 0.07–0.95; adjusted $p = 0.043$).

Considering the clinical parameter survival after relapse, only the rs11656130 genetic variant in the *MAP2K6* gene showed a significant association ($p = 0.027$). However, in the multivariate analysis this association did not remain statistically significant.

Genetic determinants and survival by localization of the tumor

In the univariate analysis, three genetic variants showed an association with RFS in patients with left colon cancer tumors: rs35251833 (*ITGAV*) ($p = 0.032$), rs833061 (*VEGFA*) ($p = 0.036$) and rs9513070 (*VEGFR1*) ($p = 0.033$) (Table 5). In the multivariate analysis, only two genetic variants retained their significance: rs9513070 of *VEGFR1* gene and rs35251833 of *ITGAV* gene. Left colon cancer patients carrying at least one G allele in the *VEGFR1* SNP showed a longer time to recurrence (HR = 0.345; 95% CI: 0.2–0.7; adjusted $p = 0.005$). The same cohort of left colon cancer patients harboring at least one G allele in the *ITGAV* variant showed an increased RFS (HR = 0.242; 95% CI: 0.1–0.6; adjusted $p = 0.005$).

Considering OS and tumor location, evaluation of the genetic variants included in the study showed a significant association with the rs9513070 variant of *VEGFR1* gene ($p = 0.031$) in left-side tumors, and with two variants in the *MAP2K6* gene: rs11656130 in right-sided tumors ($p = 0.026$) and rs2716191 in left-sided tumors ($p = 0.029$). Table 5 shows these results and the mode of inheritance. In the multivariate analysis, only the *VEGFR1* variant retained its significance. Left colon cancer patients carrying atleast one G allele in the *VEGFR1* SNP showed a longer overall survival (HR = 0.061; 95% CI: 0.01–0.5); adjusted $p = 0.011$).

Discussion

We investigated the role of germline polymorphisms as prognostic factors in stages II and III colon cancer patients in several genes involved in the angiogenesis pathway. We found that several genetic variants in the *VEGFA* and its main receptors, *VEGFR1* and *VEGFR2*, were associated with RFS and/or OS and that these associations were influenced by tumor stage and location. Several polymorphisms in additional genes of the VEGF pathway such as *MAP2K6*, *KRAS, PRKCE* and *ITGAV*, as well as in *HIFα*, a protein that stimulates the synthesis of VEGFA, and in the metastasis suppressor *KISS1*, were also associated with survival. However, only rs9513070 in the

Table 5 Germline polymorphisms in the VEGF pathway associated with RFS/OS by localization of the tumor

Recurrence free survival								
		Left colon			Right colon			
Polymorphism	*N*	Probability ± s.e. of 3-year free recurrence	Hazard ratio (95% CI)	*P*-value	*N*	Probability ± s.e. of 3-year free recurrence	Hazard ratio (95% CI)	*P*-value
ITGAV rs35251833								
G/G	100	0.858 ± 0.035	1 (reference)	0.099	56	0.721 ± 0.061	1 (reference)	0.081
G/A	86	0.820 ± 0.042	1.05 (0.5, 2.0)		74	0.875 ± 0.039	0.43 (0.2, 0.9)	
A/A	17	0.637 ± 0.119	2.58 (1.0, 6.5)		12	0.917 ± 0.080	0.50 (0.1, 2.2)	
A/A[b]	17	0.637 ± 0.119	2.52 (1.1, 6.0)	**0.032**	12	0.917 ± 0.080	0.75 (0.2, 3.2)	0.696
VEGFA rs833061								
T/T	52	0.848 ± 0.050	1 (reference)	0.111	45	0.883 ± 0.049	1 (reference)	0.199
T/C	97	0.852 ± 0.036	0.99 (0.4, 2.2)		64	0.778 ± 0.053	2.26 (0.9, 5.7)	
C/C	54	0.747 ± 0.061	1.92 (0.8, 4.3)		33	0.809 ± 0.071	1.54 (0.5, 4.8)	
C/C[b]	54	0.747 ± 0.061	1.92 (1.0, 3.6)	**0.036**	33	0.809 ± 0.071	0.90 (0.4, 2.2)	0.824
VEGFR1 rs9513070								
A/A	59	0.723 ± 0.059	1 (reference)	**0.033**	53	0.807 ± 0.055	1 (reference)	0.733
A/G[a]	96	0.864 ± 0.029	0.52 (0.3, 0.9)		69	0.824 ± 0.041	1.07 (0.5, 2.3)	
G/G[a]	47				29			
Overall survival								
		Left colon			Right colon			
Polymorphism	*N*	Probability ± s.e. of 5-year survival	Hazard ratio	*P*-value (95% CI)	*N*	Probability ± s.e. of 5-year survival	Hazard ratio	*P*-value (95% CI)
MAP2K6 rs11656130								
T/T	62	0.934 ± 0.038	1 (reference)	0.604	37	0.847 ± 0.080	1 (reference)	**0.026**
T/G	98	0.891 ± 0.051	1.61 (0.7, 3.5)		73	0.901 ± 0.004	0.88 (0.3, 2.2)	
G/G	43	0.769 ± 0.145	1.61 (0.6, 3.9)		32	0.621 ± 0.154	1.94 (0.7, 5.1)	
MAP2K6 rs2716191								
T/T	53	0.778 ± 0.104	1 (reference)	**0.029**	26	0.761 ± 0.099	1 (reference)	0.458
T/C	107	0.942 ± 0.035	0.76 (0.4, 1.5)		77	0.899 ± 0.036	0.83 (0.3, 2.1)	
C/C	43	0.869 ± 0.083	0.60 (0.2, 1.5)		39	0.708 ± 0.135	0.73 (0.2, 2.2)	
VEGFR1 rs9513070								
A/A	59	0.799 ± 0.075	1 (reference)	**0.031**	53	0.828 ± 0.078	1 (reference)	0.525
A/G[a]	96	0.924 ± 0.040	0.061 (0.01, 0.5)		69	0.837 ± 0.049	1.34 (0.4, 4.4)	
G/G[a]	47				29			

P-value was based on log-rank test in codominant

s.e. Greedwood standard error

[a] Dominant model

[b] Recessive model

The bold values indicate the *P*-values statistically significant ($P < 0.05$)

VEGFR1 gene, rs1137282 in the *KRAS* gene and rs35251833 in the *ITGAV* gene retained their significance after multivariate adjustments.

In the present work, we discuss only those published studies that have identified polymorphisms in VEGF-related genes as predictive biomarkers for tumor recurrence in stages II or III colon cancer patients. We do not discuss data from case-control studies.

In 2008, Lurje et al. studied 10 polymorphisms in eight genes involved in the angiogenesis pathway in a cohort of 125 stage III CC patients [5]. They demonstrated that patients with the T allele in rs3025039 (+ 936 C/T), located in the VEGFA gene, showed a significantly longer time to recurrence (TTR) ($p = 0.003$). However, they found no correlation between rs2010963 (−634 G/C), also located in the *VEGFA* gene, and TTR. Some time later, these same

authors also analyzed both polymorphisms in a cohort of 109 stage II colon cancer patients [23]. Surprisingly, they found a contradictory result: rs3025039 was not associated with TTR, whereas rs2010963 was significantly associated with this outcome parameter ($p = 0.028$). They also analyzed rs1870377 of *VEGFR2*, finding no association between this polymorphism and TTR. More recently, Kjaer-Frifeldt et al. studied 3 polymorphisms in the VEGFA gene in a cohort of 698 stage II colon cancer patients. They found that rs699947 and rs833061 were significantly associated with TTR ($p = 0.02$ for both SNPs), whereas rs2010963 was not [24]. It should be emphasized that these studies evaluated a reduced number of SNPs in VEGF-related genes in a cohort of stages II and III colon cancer patients and that their results are contradictory. For this reason we designed a study that included functional SNPs of several VEGF-related genes, focusing especially on the ligands *(VEGFA)*, receptors (*VEGFR1* and *VEGFR2)*, and other relevant genes in the VEGF signal cascade.

One of the results of the present study concerns rs9513070, a SNP located in the *VEGFR1* gene. To the best of our knowledge, this is the first study that shows that the variant rs9513070 is associated with survival in both stage II CC and in left-sided primary tumor location. Tumor sidedness has a great impact on prognosis, as right and left colon cancer tumors harbor different biological features. Whereas right-sided tumors are more likely to present microsatellite instability, mutations in *KRAS* or *BRAF* or *MLH1* methylation, left-sided tumors are associated with CIN, p53 or miRNAs [25–29]. Additionally, right colon cancers present a higher expression of pro-angiogenic factors, which justifies the better response to bevacizumab of these tumors [30]. Interestingly, rs9513070 variant had been associated with survival in a previous study conducted in advanced CC patients [21]. In that study, the median PFS and OS were superior in patients with the VEGFR1 rs9513070 A/A genotype (8.7 vs. 6.6 months; $p = 0.001$ and 26.4 vs. 16.1 months; $p = 0.038$, respectively). Expression studies conducted by Ning et al. showed that VEGF and VEGFR1 gene expression levels were significantly associated with TTR. One of their studies included 140 stage II–III CC patients treated with adjuvant chemotherapy [31]. They observed that patients with lower VEGF or VEGFR1 gene expression levels had longer time to recurrence than those with higher expression levels of these genes ($p < 0.05$, log-rank test). All these data, along with the results of the in silico analysis of this intron variant (http://compbio.cs.queensu.ca/F-SNP), strengthen the hypothesis that VEGFR1 could play a role in CC outcomes. However, until the mechanisms underlying all these findings are not fully elucidated an independent validation study that confirms our results is needed to highlight the clinical relevance of *VEGFR1* polymorphisms.

Regarding the association between rs1137282 in the *KRAS* gene and OS, we observed that stage II patients harboring a homozygous GG genotype were significantly associated with a decreased OS. Our group recently conducted a study in 131 patients who underwent surgery for stage I-III non-small cell lung cancer. We found that the GG genotype was also associated with a poor prognosis [17]. In vitro experiments have demonstrated that the minor allele (G) increase luciferase activity significantly and in silico analyses predict that this SNP introduces an exon splicing enhancer motif and disrupts an exon splicing silencer motif [12]. These findings reinforce the role of this SNP as a prognostic biomarker in cancer.

Finally, the present study shows that rs35251833 SNP in the *ITGAV* gene correlated with RFS in left CC patients: G/G and G/A genotypes were significantly associated with an increased RFS. This variant is in high linkage-disequilibrium with rs1839123 that showed similar results in a previous study conducted by our group in metastatic colorectal cancer patients [14]. Bohanes et al. studied the role of genetic variants in several integrin genes and although they reported some associations in stages II–III CC, the rs35251833 was not included in their study [32]. In silico analysis of this intronic SNP suggests a potential role in transcriptional regulation (http://compbio.cs.queensu.ca/F-SNP).

In conclusion, this study provides evidence that germline polymorphisms in *VEGFR1*, *KRAS* and *ITGAV* genes are associated with prognosis in stages II–III CC patients. As stage and tumor location have also been correlated with prognosis, future genetic studies should stratify CC patients according to these parameters.

Acknowledgements This work was supported by the Departament d'Economia i Coneixement de la Generalitat de Catalunya (2016FI_B 00368 to Pau Riera).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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