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Original Research

Autophagy-related polymorphisms predict hypertension in patients with metastatic colorectal cancer treated with FOLFIRI and bevacizumab: Results from TRIBE and FIRE-3 trials

Martin D. Berger [a], Shinichi Yamauchi [a], Shu Cao [b], Diana L. Hanna [a], Yu Sunakawa [a], Marta Schirripa [a,d], Satoshi Matsusaka [a], Dongyun Yang [b], Susan Groshen [b], Wu Zhang [a], Yan Ning [a], Satoshi Okazaki [a], Yuji Miyamoto [a], Mitsukuni Suenaga [a], Sara Lonardi [d], Chiara Cremolini [e], Alfredo Falcone [e], Volker Heinemann [c], Fotios Loupakis [d], Sebastian Stintzing [c], Heinz-Josef Lenz [a,b,*]

[a] *Division of Medical Oncology, Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, 1441 Eastlake Avenue, Los Angeles, CA 90033, USA*
[b] *Department of Preventive Medicine, Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, 1441 Eastlake Avenue, Los Angeles, CA 90033, USA*
[c] *Department of Medical Oncology and Comprehensive Cancer Center, University of Munich (LMU), Marchioninistrasse 15, 81377 Munich, Germany*
[d] *Oncologia Medica 1, Istituto Oncologico Veneto, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS), Via Gattamelata 64, 35128 Padova, Italy*
[e] *U.O. Oncologia Medica, Azienda Ospedaliero-Universitaria Pisana, Istituto Toscano Tumori, Via Roma 67, 56126 Pisa, Italy*

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KEYWORDS
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Abstract ***Purpose:*** The most frequent bevacizumab-related side-effects are hypertension, proteinuria, bleeding and thromboembolism. To date, there is no biomarker that predicts anti-VEGF–associated toxicity. As autophagy inhibits angiogenesis, we hypothesised that single-nucleotide polymorphisms (SNPs) within autophagy-related genes may predict bevacizumab-mediated toxicity in patients with metastatic colorectal cancer (mCRC).

* *Corresponding author*: Division of Medical Oncology, University of Southern California, Norris Comprehensive Cancer Center, Keck School of Medicine, 1441 Eastlake Avenue, Los Angeles, CA 90033, USA. Fax: +1 323 865 0061.
E-mail address: lenz@med.usc.edu (H.-J. Lenz).

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polymorphism; Bevacizumab-associated toxicity; FOLFIRI/ bevacizumab

Patients and methods: Patients with mCRC treated with first-line FOLFIRI and bevacizumab in two phase III randomised trials, namely the TRIBE trial (n = 219, discovery cohort) and the FIRE-3 trial (n = 234, validation cohort) were included in this study. Patients receiving treatment with FOLFIRI and cetuximab (FIRE-3, n = 204) served as a negative control. 12 SNPs in eight autophagy-related genes (ATG3/5/8/13, beclin 1, FIP200, unc-51-like kinase 1, UVRAG) were analysed by PCR-based direct sequencing.

Results: The FIP200 rs1129660 variant showed significant associations with hypertension in the TRIBE cohort. Patients harbouring any G allele of the FIP200 rs1129660 SNP showed a significantly lower rate of grade 2–3 hypertension compared with the A/A genotype (3% versus 15%, odds ratio [OR] 0.17; 95% confidence interval [CI], 0.02–0.73; $P = 0.009$). Similarly, G allele carriers of the FIP200 rs1129660 SNP were less likely to develop grade 2–3 hypertension than patients with an A/A genotype in the FIRE-3 validation cohort (9% versus 20%, OR 0.43; 95% CI, 0.14–1.11; $P = 0.077$), whereas this association could not be observed in the control cohort (12% versus 9%, OR 1.40; 95% CI, 0.45–4.04; $P = 0.60$).

Conclusion: This is the first report demonstrating that polymorphisms in the autophagy-related FIP200 gene may predict hypertension in patients with mCRC treated with FOLFIRI and bevacizumab.

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1. Introduction

The cornerstone of first-line palliative treatment in patients with metastatic colorectal cancer (mCRC) consists of 5-fluorouracil-based chemotherapy, in combination with either oxaliplatin (FOLFOX) or irinotecan (FOLFIRI) [1]. The addition of bevacizumab, an antibody that binds circulating vascular endothelial growth factor-A, has led to improved response rates, progression-free and overall survival [1]. Bevacizumab has also improved outcomes in the second-line and maintenance settings, and is used in patients with liver-limited mCRC eligible for curative resection [2–4]. The most frequent adverse events of bevacizumab include hypertension, proteinuria, bleeding, thromboembolism and impaired wound healing [1]. Importantly, hypertension and proteinuria have been considered on-target effects of anti-angiogenic therapy and proposed as predictive markers of efficacy in mCRC [5], but the underlying mechanisms are not well understood.

Autophagy plays an important role in cancer, cardiovascular disease, and angiogenesis [6–8] and regulates cellular homoeostasis by facilitating lysosomal degradation of dysfunctional cytoplasmatic proteins [9]. These degradation products, in turn, serve as metabolic precursors for the synthesis of new cellular constituents. A finely tuned regulation of autophagy is mandatory to maintain an equilibrium between cell survival and cell death. The relationship between autophagy and malignancy is complex, and autophagic mechanisms have been shown to be both oncogenic and tumour suppressive in different cancers [6,10]. Previous studies have also linked autophagy with hypertension, thrombosis and haemostasis [7,11], and other vascular disorders [12]. Furthermore, there is accumulating evidence that anti-angiogenics can induce autophagy [13], and that autophagy inhibits angiogenesis and may potentiate the effects of anti-angiogenic therapy [13,14].

The literature regarding genetic polymorphisms and bevacizumab-mediated toxicity is scarce, with a few studies demonstrating an association between single-nucleotide polymorphisms (SNPs) within the vascular endothelial growth factor pathway and hypertension [15,16]. We hypothesised that variants within autophagy-related genes may be associated with hypertension, proteinuria, bleeding or venous thromboembolism in mCRC patients enrolled in the phase III TRIBE and FIRE-3 trials and assigned to first-line FOLFIRI and bevacizumab.

12 SNPs in eight autophagy-related genes were examined in this study (autophagy-related protein 13 [ATG13], ATG3, ATG5, ATG8, beclin 1 [BECN1], focal adhesion kinase family interacting protein of 200 kDa [FIP200], unc-51-like kinase 1 [ULK1] and UV radiation resistance-associated gene protein [UVRAG]).

2. Material and methods

2.1. Study design and patient population

A total of 657 patients with unresectable mCRC enrolled in the randomised phase III TRIBE and FIRE-3 trials and treated with first-line therapy with FOLFIRI and bevacizumab or FOLFIRI and cetuximab were included in this study [17,18]. The discovery cohort consisted initially of 228 patients receiving FOLFIRI and bevacizumab (TRIBE) and the validation cohort comprised 247 patients in FIRE-3 (FOLFIRI bevacizumab arm). Patients treated with FOLFIRI and

cetuximab within the FIRE-3 trial served as a negative control (n = 226). A flow chart illustrates the selection of the final study cohorts (Supplementary Fig. S1). The study was approved by the local ethics committees for each participating site. All patients provided informed consent for molecular analyses. Molecular analyses were conducted at the University of Southern California (Norris Comprehensive Cancer Center). Our study was conducted adhering to the reporting recommendations for tumour marker prognostic studies [19]. Side-effects were reported according to the Common Terminology Criteria for Adverse Events (version 3.0) [20]. Management of toxicities and detailed dosage information for the therapeutic regimens in each treatment arm are described in Supplementary material S1.

2.2. Candidate polymorphisms

SNPs of genes involved in autophagy were selected according to the following criteria: minor allele frequency ≧10% in Caucasians; potential to alter gene function in a biologically relevant matter based on public databases (www.snpinfo.niehs.nih.gov, www.compbio.cs.queensu.ca, www.ncbi.nlm.nih.gov).

2.3. Genotyping

Genomic DNA was extracted from peripheral blood (discovery cohort) and formalin-fixed paraffin-embedded tissue (validation and control group) using the QIAmp kit (Qiagen, USA). Candidate SNPs were tested using polymerase chain reaction (PCR)-based direct DNA sequence analysis. Briefly, forward and reverse primers were used for PCR amplification (Supplementary Table S1). PCR fragments were sequenced on an ABI 3100A Capillary Genetic Analyzer (Applied Biosystem, Foster City, USA) and analysed in either sense or anti-sense direction to detect the SNP.

2.4. Statistical analysis

The aim of this study was to identify SNPs within autophagy-related genes associated with bevacizumab-related toxicities such as hypertension, proteinuria, bleeding and venous thromboembolism. Due to the low incidence, we did not include grade 2–3 bleeding in our analysis. Allelic distribution of polymorphisms was tested for deviation from Hardy–Weinberg equilibrium (HWE) using a chi-square test. Differences in the baseline characteristics of the three cohorts were compared by using the chi-square test for categorical factors and the Kruskal–Wallis test for numeric variables.

To evaluate the effects of different SNPs on toxicities in the discovery cohort, exact logistic regression was applied for each SNP in univariable- and multivariable models [21], adjusted for age, sex and body mass index. *P* values were calculated based on exact conditional score test. We then constructed a multivariable exact logistic model to determine the best combination of SNPs to predict toxicity. SNPs, which significantly predicted toxicity, were included in the final model controlling for the same covariates. In a next step, statistically significant toxicity-related SNPs in the discovery cohort were tested in the validation cohort (FIRE-3 FOLFIRI bevacizumab arm).

Patients enrolled in the FIRE-3 FOLFIRI cetuximab arm served as a negative control. Power analyses for the three cohorts are shown in Supplementary material S1. SAS 9.4 was used to perform all analyses. All tests were two-sided at a significance level of 0.05. PASS 2008 was used for power analysis.

3. Results

The baseline characteristics of the study population comprising the discovery (TRIBE trial) and validation/control cohorts (FIRE-3 trial) are outlined in Table 1. Genotyping was successful in >94% of the cases for each polymorphism. Causes of failure were due to limited quality or quantity of extracted genomic DNA. All tested SNPs were within the Hardy–Weinberg equilibrium.

As depicted in Table 2, in univariate analysis, any C allele carriers of the ATG13 rs13448 SNP showed a significantly lower rate of grade 2–3 hypertension compared with the homozygous T/T genotype (4% versus 15%, odds ratio [OR], 0.22; 95% confidence interval (CI), 0.04–0.78; $P = 0.012$). This association was confirmed in the multivariable model (OR, 0.24; 95% CI, 0.04–0.84; $P = 0.020$). Similarly, patients harbouring any G allele of FIP200 rs1129660 had less-frequent grade 2 or 3 hypertension than those carrying the homozygous A/A genotype (A/G or G/G [3%] versus A/A [15%], OR, 0.17; 95% CI, 0.02–0.73; $P = 0.009$). This association was also confirmed in multivariable analysis (OR, 0.17; 95% CI, 0.02–0.78; $P = 0.014$). In addition, patients with an A/G or A/A genotype of ULK1 rs9481 showed a lower incidence of grade 2 or 3 hypertension (A/G or A/A [4%] versus G/G [13%], OR, 0.25; 95% CI, 0.03–1.05; $P = 0.047$).

There was a trend towards a lower incidence of grade 2–3 proteinuria in patients carrying any G allele of FIP200 rs17337252 A/A genotype (any G [7%] versus A/A [16%], OR, 0.38; 95% CI, 0.13–1.11; $P = 0.056$, adjusted $P = 0.065$). Similarly, patients harbouring the A/A genotype of BECN1 rs11552192 had a tendency towards fewer grade 3–4 VTE, compared with those harbouring any T allele (A/T or T/T) (5% versus 14%, OR, 2.98; 95% CI, 0.74–10.71; $P = 0.066$, adjusted $P = 0.060$) (Supplementary Table S2).

Three SNPs were identified to be significantly associated with hypertension, namely ATG13 rs13448, FIP200 rs1129660 and ULK1 rs9481. They were then included in a multivariable model adjusting for age, sex

Table 1
Comparisons of baseline clinical characteristics of patients between TRIBE FOLFIRI bevacizumab, FIRE-3 FOLFIRI bevacizumab and FIRE-3 FOLFIRI cetuximab arms.

Patient characteristics	TRIBE FOLFIRI bevacizumab (n = 219)		FIRE-3 FOLFIRI bevacizumab (n = 234)		FIRE-3 FOLFIRI cetuximab (n = 204)		P value[a]
	n	%	n	%	n	%	
Sex							0.13
Male	134	61.2	158	67.5	143	70.1	
Female	85	38.8	76	32.5	61	29.9	
Age (years)							<0.001
Median (range)	60 (29–75)		65 (31–76)		64 (38–76)		
<65	147	67.1	120	51.3	102	50.0	
≥65	72	32.9	114	48.7	102	50.0	
BMI							0.12
Median (range)	25 (14–39)		25 (13–41)		25 (15–45)		
<25	113	53.3	110	47.0	97	47.8	
≥25	99	46.7	124	53.0	106	52.2	
Performance status							<0.001
ECOG 0	183	83.6	125	53.4	107	52.4	
ECOG 1–2	35	16.0	109	46.6	97	47.6	
Unknown[b]	1	0.4	–		–		
Tumour site							0.092
Right-sided colon	54	24.7	58	24.8	36	17.6	
Left-sided colon	151	68.9	170	72.6	164	80.4	
Unknown[b]	14	6.4	6	2.6	4	2.0	
Liver metastasis							0.76
Yes	71	32.4	79	33.8	73	35.8	
No	148	67.6	155	66.2	131	64.2	
Number of metastatic sites							0.97
<2	97	44.3	101	43.2	89	43.6	
≥2	122	55.7	133	56.8	115	56.4	
Primary tumour resection							<0.001
Yes	140	63.9	203	86.8	169	82.8	
No	79	36.1	31	13.2	33	16.2	
Unknown[b]	–		–		2	1.0	
High LDH							0.55
Yes	101	46.1	68	29.1	58	28.4	
No	86	39.3	70	29.9	62	30.4	
Unknown[b]	32	14.6	96	41.0	84	41.2	
Adjuvant chemotherapy							0.030
Yes	26	11.9	43	18.4	43	21.1	
No	193	88.1	191	81.6	159	77.9	
Unknown[b]	–		–		2	1.0	
Time to metastasis							0.093
Synchronous	181	82.6	175	74.8	151	74	
Metachronous	38	17.4	58	24.8	50	24.5	
Unknown[b]	–		1	0.4	3	1.5	
KRAS status							–
Wildtype	92	42.0	234	100	204	100	
Mutant	90	41.1	–		–		
Unknown[b]	37	16.9	–		–		
BRAF status							0.22
Wildtype	172	78.5	203	86.8	180	88.2	
Mutant	10	4.6	23	9.8	18	8.8	
Unknown[b]	37	16.9	8	3.4	6	2.9	

[a] P value was based on chi-square test for categorical factors and the Kruskal–Wallis test for numeric variables.
[b] Unknown group was not included in the analysis.

and body mass index (Table 3). ATG13 rs13448 and FIP200 rs1129660 remained significant (adjusted P = 0.017 and 0.011, respectively). However, ULK1 rs9481 became insignificant (adjusted P = 0.092).

Next, we prospectively tested these two SNPs and their association with hypertension in the validation cohort.

Whereas the significantly lower rate of grade 2–3 hypertension among C allele carriers of the ATG13

Table 2
Associations between autophagy-related SNPs and hypertension in the discovery cohort (TRIBE FOLFIRI bevacizumab).

SNP	*n*	Hypertension		Univariate exact logistic regression		Multivariable exact logistic regression[a]	
		0–1	*2–3*	Exact odds ratio (95% CI)	*P* value[b]	Exact odds ratio (95% CI)	*P* value[b]
ATG13rs13448					**0.012**		**0.020**
T/T	139	118	21	1 (Reference)		1 (Reference)	
Any C	80	77	3	0.22 (0.04, 0.78)		0.24 (0.04, 0.84)	
ATG3rs9831088					0.29		0.27
A/A	112	97	15	1 (Reference)		1 (Reference)	
Any G	105	96	9	0.61 (0.22, 1.57)		0.57 (0.20, 1.50)	
ATG5rs633724					1.00		0.83
C/C	99	88	11	1 (Reference)		1 (Reference)	
Any T	116	103	13	1.01 (0.40, 2.63)		1.12 (0.43, 3.01)	
ATG8rs11149841					0.25		0.24
G/G	148	129	19	1 (Reference)		1 (Reference)	
Any T	69	64	5	0.53 (0.15, 1.56)		0.49 (0.13, 1.50)	
ATG8rs8060972					0.61		0.43
A/A	164	148	16	1 (Reference)		1 (Reference)	
Any T	53	46	7	1.41 (0.46, 3.89)		1.53 (0.49, 4.35)	
BECN1rs11552192					0.59		0.77
A/A	182	161	21	1 (Reference)		1 (Reference)	
Any T	37	34	3	0.68 (0.12, 2.47)		0.72 (0.13, 2.67)	
FIP200rs1129660					**0.009**		**0.014**
A/A	149	127	22	1 (Reference)		1 (Reference)	
Any G	70	68	2	0.17 (0.02, 0.73)		0.17 (0.02, 0.78)	
FIP200rs17337252					1.00		1.00
A/A	56	50	6	1 (Reference)		1 (Reference)	
Any G	163	145	18	1.03 (0.37, 3.37)		1.03 (0.36, 3.38)	
ULK1rs11616018					1.00		0.82
T/T	151	134	17	1 (Reference)		1 (Reference)	
Any C	68	61	7	0.91 (0.30, 2.45)		0.87 (0.28, 2.44)	
ULK1rs12303764					0.37		0.35
T/T	79	68	11	1 (Reference)		1 (Reference)	
Any G	139	126	13	0.64 (0.25, 1.67)		0.65 (0.24, 1.75)	
ULK1rs9481					**0.047**		0.069
G/G	164	142	22	1 (Reference)		1 (Reference)	
Any A	55	53	2	0.25 (0.03, 1.05)		0.23 (0.02, 1.04)	
UVRAGrs1458836					0.27		0.26
C/C	178	156	22	1 (Reference)		1 (Reference)	
Any T	41	39	2	0.37 (0.04, 1.59)		0.39 (0.04, 1.71)	

Bold indicates statistical significant *P* values.
[a] Multivariable model was adjusted for sex (female versus male), age (<65 versus ≥65) and BMI (<25 versus ≥25).
[b] *P* value was based on exact conditional scores test from exact logistic regression.

Table 3
Multivariable exact logistic regression model: SNPs within autophagy-related genes predict grade 2–3 hypertension according to genotype (discovery cohort).

SNP	*n*	Hypertension		Multivariable exact logistic regression[a]	
		0–1	*2–3*	Exact odds ratio (95% CI)	*P* value[b]
ATG13rs13448					**0.017**
T/T	139	118 (85%)	21 (15%)	1 (Reference)	
Any C	80	77 (96%)	3 (4%)	0.23 (0.04, 0.83)	
FIP200rs1129660					**0.011**
A/A	149	127 (85%)	22 (15%)	1 (Reference)	
Any G	70	68 (97%)	2 (3%)	0.17 (0.02, 0.77)	
ULK1rs9481					0.092
G/G	164	142 (87%)	22 (13%)	1 (Reference)	
Any A	55	53 (96%)	2 (4%)	0.25 (0.03, 1.20)	

Bold indicates statistical significant *P* values.
[a] Multivariable model includes all significant SNPs in the univariate model (Table 2), adjusted for sex (female versus male), age (<65 versus ≥65) and BMI (<25 versus ≥25).
[b] *P* value was based on exact conditional scores test from exact logistic regression.

rs13448 SNP observed in the discovery cohort (see above) could not be shown in the validation cohort (18% versus 16%, OR 1.13, 95% CI, 0.51–2.63; $P = 0.85$), G allele carriers of the FIP200 rs1129660 SNP in the validation cohort still exhibited a trend towards the lower incidence of grade 2 or 3 hypertension (9% versus 20%, OR 0.43, 95% CI, 0.14–1.11; $P = 0.077$, adjusted $P = 0.072$). However, this association could not be seen in patients treated with FOLFIRI and cetuximab within the control cohort (Table 4).

Due to the low incidence of grade 2–3 hypertension among G allele carriers of the FIP200 rs1129660 SNP in both the discovery and validation cohorts, we also conducted an analysis combining the patients of the FIRE-3 and TRIBE cohorts. Patients carrying any G allele of FIP200 rs1129660 SNP showed still a lower rate of grade 2–3 hypertension (6% versus 17%) compared with those with an A/A genotype in univariate (OR 0.30, 95% CI,

Table 4
Association between autophagy-related SNPs and hypertension in the validation and control cohorts.

SNP	*n*	Hypertension		Univariate exact logistic regression		Multivariable exact logistic regression[a]	
		0–1	*2–3*	Exact odds ratio (95% CI)	*P* value[b]	Exact odds ratio (95% CI)	*P* value[b]
Validation cohort (FIRE-3 bevacizumab arm)							
ATG13rs13448					0.85		0.85
T/T	74	62 (84%)	12 (16%)	1 (Reference)		1 (Reference)	
Any C	145	119 (82%)	26 (18%)	1.13 (0.51, 2.63)		1.13 (0.50, 2.67)	
FIP200rs1129660					0.077		0.072
A/A	167	134 (80%)	33 (20%)	1 (Reference)		1 (Reference)	
Any G	63	57 (91%)	6 (9%)	0.43 (0.14, 1.11)		0.41 (0.13, 1.07)	
Control cohort (FIRE-3 cetuximab arm)							
FIP200rs1129660					0.60		0.60
A/A	146	133 (91%)	13 (9%)	1 (Reference)		1 (Reference)	
Any G	58	51 (88%)	7 (12%)	1.40 (0.45, 4.04)		1.38 (0.44, 3.97)	

[a] Multivariable model was adjusted for sex (female versus male), age (<65 versus ≥65) and BMI (<25 versus ≥25).
[b] *P* value was based on exact conditional scores test from exact logistic regression.

Table 5
Association between autophagy-related SNPs and hypertension by combining TRIBE FOLFIRI bevacizumab and FIRE-3 FOLFIRI bevacizumab arms.

SNP	*n*	Hypertension		Univariate exact logistic regression		Multivariable exact logistic regression[a]	
		0–1	*2–3*	Exact odds ratio (95% CI)	*P* value[b]	Exact odds ratio (95% CI)	*P* value[b]
ATG13rs13448					0.49		0.40
T/T	213	180 (85%)	33 (15%)	1 (Reference)		1 (Reference)	
Any C	225	196 (87%)	29 (13%)	0.81 (0.45, 1.43)		0.79 (0.44, 1.41)	
FIP200rs1129660					**0.002**		**0.001**
A/A	316	261 (83%)	55 (17%)	1 (Reference)		1 (Reference)	
Any G	133	125 (94%)	8 (6%)	0.30 (0.12, 0.67)		0.29 (0.12, 0.66)	

Bold indicates statistical significant *P* values.
[a] Multivariable model was adjusted for sex (female versus male), age (<65 versus ≥65) and BMI (<25 versus ≥25).
[b] *P* value was based on exact conditional scores test from exact logistic regression.

0.12–0.67; $P = 0.002$) and multivariable analyses (OR 0.29, 95% CI, 0.12–0.66; $P = 0.001$) (Table 5).

4. Discussion

To the best of our knowledge, our study is the first to analyse SNPs in autophagy-related genes and their association with bevacizumab-related toxicities in patients with mCRC treated with FOLFIRI and bevacizumab. With the introduction of targeted drugs into the treatment algorithm of mCRC, the prognosis of patients has significantly improved, and the median overall survival now exceeds 30 months [22]. As bevacizumab and other anti-angiogenics are integrated into second and later treatment lines [3], the total exposure and risk for toxicity has increased [23]. It is therefore crucial to identify biomarkers that predict bevacizumab-related adverse effects in patients with mCRC.

Autophagy is a highly conserved catabolic pathway which aims to maintain cell function by degrading damaged organelles and allows cells to survive under the stresses of hypoxia, nutrient deprivation, inflammation or other cytotoxic insults. Anti-angiogenic therapy results in sustained oxygen deprivation which may upregulate autophagy-related genes aimed at preserving cell survival [24]. In addition, in glioma cell lines, Liang *et al.* demonstrated that bevacizumab initiates autophagy independent of hypoxia [25].

Moreover, autophagy plays a crucial role in maintaining vascular integrity and assumes a close relationship with angiogenesis. Guo *et al.* demonstrated that steady laminar shear stress stimulates autophagy and endothelial nitric oxide synthase, whereas pretreatment with an autophagy inhibitor (3-methyladenine) inhibits endothelial nitric oxide synthase expression. These findings illustrate that autophagy is essential for regulating endothelial cell function and vascular tone [26]. There is also growing evidence that autophagy may attenuate the detrimental effects of glucose-induced endothelial damage in patients with diabetes mellitus and reduce angiotensin II-related endothelial injury in patients with hypertension [27,28]. Autophagy has been implicated in the pathogenesis of atherosclerosis and vasculitis, suggesting a potential role in bevacizumab-associated toxicities, such as hypertension, proteinuria and thromboembolism.

This study provides the first evidence that SNPs in genes involved in autophagy, namely ATG13 rs13448, FIP200 rs1129660, and ULK1 rs9481 might be associated with grade 2–3 hypertension in mCRC patients

treated with first-line FOLFIRI and bevacizumab. However, ULK1 rs9481 and its association with hypertension could not withstand multivariable analysis.

ULK1, a serine/threonine protein kinase plays a critical role in the initiation of autophagy [29]. Under nutrient-poor conditions, dissociation of mTOR stimulates ULK1 which phosphorylates ATG13 and FIP200 to induce autophagy and subsequent angiogenesis inhibition [30,31].

Previous studies demonstrated that both ATG13 and FIP200 knockout mice embryos showed early lethality and thinning of the ventricular wall [32,33] due to increased apoptosis of cardiomyocytes. Interestingly, both cardiomyocytes and peripheral vascular smooth muscle cells derive from precardiac mesoderm and share common precursor cells, namely the bipotential myogenic progenitors [34,35]. One may, therefore, assume that FIP200 and ATG13 exert not only an essential role in cardiac development but also in peripheral vascular morphogenesis and functionality. However, this hypothesis warrants further confirmation in future experimental models.

We believe that genetic variants of ATG13 and FIP200 may differently influence vascular integrity thus leading to a varying susceptibility to developing hypertension.

Although the underlying molecular and pathophysiological mechanism of how FIP200 and ATG13 exactly influence development of hypertension still remains unclear at present, our findings obtained from three cohorts in two independent randomised phase III trials provide a strong rationale for further investigations in this field.

Recently, Long *et al.* demonstrated that the expression of autophagy-related genes was upregulated in murine models of pulmonary arterial hypertension [36]. Furthermore, they showed that treatment with chloroquine was associated with inhibition of proliferation and induction of apoptosis in pulmonary artery smooth muscle cells [36]. In another pre-clinical model, drugs inhibiting autophagy in the rostral ventral medulla resulted in decreased hypertension [37]. These and our findings suggest a major role of autophagy in the pathogenesis of hypertension.

ATG13 rs13448 is a three prime UTR variant located on chromosome 11. By providing binding sites for microRNAs such as miR-146a, miR-146-5p, miR-34b, miR-562, miR-589, and miR-646, this genetic variant regulates gene expression at post-transcriptional levels. FIP200 rs1129660 is located on chromosome 8 and modulates exonic splicing [38]. However, the exact function of these SNPs different genotypes on gene expression still remains to be elucidated.

There are several reasons why we intentionally restricted our analysis to patients who were uniformly treated with FOLFIRI and bevacizumab and not FOLFOXIRI bevacizumab.

First, in contrast to patients treated with irinotecan, those receiving oxaliplatin have in general, independently from concomitant bevacizumab treatment, a small but appreciable risk to develop hypertension, thromboembolic events and proteinuria (1–10%). Accordingly, in the TRIBE trial grade 3/4 hypertension occurred twice as often in patients treated with FOLFOXIRI and bevacizumab than in those receiving FOLFIRI and bevacizumab, however without reaching statistical significance [17]. Second, there are preliminary data showing an interaction of autophagy-related proteins (ATG) with oxaliplatin [39], whereas no association of these ATG with irinotecan has been described so far.

Due to these differences and to avoid an imbalanced comparison due to additional oxaliplatin exposure in one group, which might introduce bias and confound our results, we did not test these SNPs in the FOLFOXIRI bevacizumab arm.

In summary, we identified two SNPs within two autophagy-related genes predicting hypertension in mCRC patients enrolled in the phase III TRIBE trial and uniformly treated with first-line FOLFIRI and bevacizumab. In a next step, we tested these two SNPs in the FOLFIRI bevacizumab arm of the phase III randomised FIRE-3 trial. Although statistically not significant, a strong trend towards a lower incidence of grade 2 and 3 hypertension among patients harbouring any G allele of FIP200 rs1129660 could be observed in the validation cohort. However, this association could not be shown in patients treated within the FOLFIRI cetuximab arm, which served as a negative control. Due to the low number of patients developing grade 2–3 hypertension in both, the discovery and the validation set, we also combined both cohorts and could demonstrate an even stronger correlation between G allele carriers of FIP200 rs1129660 and lower rate of high-grade hypertension.

The association of the ATG13 rs13448 SNP with grade 2 and 3 hypertension disappeared and could not be validated in the FIRE-3 cohort.

The limitations of the study are the small number of patients with high-grade toxicities and that information about existing anti-hypertensive therapy is lacking. Furthermore, we did not provide an exact mechanism of how various genotypes of the FIP200 rs1129660 SNP differently influence grade of hypertension. Hence, more basic biology research is warranted to further elucidate the role of autophagy in bevacizumab-associated hypertension.

In conclusion, our study suggests a significance of FIP200 rs1129660 SNP in predicting grade 2–3 hypertension in mCRC patients treated with FOLFIRI and bevacizumab. Patients harbouring an A/A genotype might derive benefit from self-measured blood pressure monitoring.

Conflict of interest statement

None declared.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejca.2017.02.020.

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