Original Article

Tumor Biology

Tumor Biology
June 2020: 1–11

DOI: 10.1177/1010428320925237
journals.sagepub.com/home/tub
SAGE

KCNB1 gene polymorphisms and related indel as predictor biomarkers of treatment response for colorectal cancer – toward a personalized medicine

Mouadh Barbirou[1,2], Ikram Sghaier[2,3], Sinda Bedoui[4], Rahma Ben Abderrazek[1], Hazar Kraiem[1], Azer Farah[1], Rym Hassiki[1], Amina Mokrani[5], Amel Mezlini[5], Wassim Y Almawi[3,6], Besma Loueslati-Yacoubi[4] and Balkiss Bouhaouala-Zahar[1,7]

Abstract

The *KCNB1* gene variants were differentially associated with cancers. However, their association with colorectal cancer has not yet been explored. We investigated the contribution of the *KCNB1* gene variants rs3331, rs1051295, and indel (insertion/deletion) rs11468831 Polymorphism as predictors of the treatment response in colorectal cancer patients. A retrospective study, which involved 291 Tunisian colorectal cancer patients (aged 60.0 ± 13.1 years), who were stratified into responder and non-responder groups, according to TNM stages and their responsiveness to chemotherapy based on fluorouracil. *KCNB1* genotyping was performed with amplification-refractory mutation system–polymerase chain reaction, and was confirmed by Sanger sequencing. Sex-specific response was found and colorectal cancer females are less likely to achieve a positive response during the chemotherapy strategy, compared to males. Weight and body mass index, tumor size, and tumor localization are considered as predictive factors to treatment responsiveness. Carriage of rs11468831 Ins allele was significantly associated with successful therapy achievement (p *adjusted* < 0.001). Stratification of colorectal cancer patients' response according to tumor localization and TNM stages reveals negative association of rs3331 Major allele to treatment response among the patients with advanced cancer stages (subgroup G2). The presence of rs3331 (homozygous minor) C/C genotype was positively associated with decline in carcinoembryonic antigen ($p = 0.043$) and CA19-9 ($p = 0.014$) serum levels. On the other hand, the presence of rs1051295 (homozygous minor) A/A genotype was correlated with marked decline in CA19-9 serum levels. *KCNB1* haplotype did not reveal any association between haplotypes and treatment response. The results obtained suggest that gender-specific strategies for screening treatment and prevention protocols as well as *KCNB1* variants may constitute an effective model for ongoing personalization medicine.

[1]Laboratory of Venoms and Therapeutic Molecules, Pasteur Institute of Tunis, Tunis Belvédère- University of Tunis El Manar, Tunis, Tunisia
[2]Department of Health Management and Informatics, Center for Biomedical Informatics, School of Medicine, University of Missouri, Columbia, MO, USA
[3]Faculty of Sciences of Tunis, University of Tunis El Manar, Tunis, Tunisia
[4]Laboratory of Mycology, Pathologies and Biomarkers, Faculty of Sciences of Tunis, University of Tunis El Manar, Tunis, Tunisia
[5]Salah Azaiz Oncology Institute, Tunis, Tunisia
[6]School of Medicine, Nazarbayev University, Nur-Sultan, Kazakhstan
[7]Medicine School of Tunis, University of Tunis El Manar, Tunis, Tunisia

Corresponding author:
Balkiss Bouhaouala-Zahar, Laboratory of Venoms and Therapeutic Molecules, Pasteur Institute of Tunis, Tunis Belvédère- University of Tunis El Manar, 13 Place Pasteur, BP74, 1007 Tunis, Tunisia.
Emails: balkiss.bouhaouala@pasteur.tn; balkiss.bouhaouala@fmt.utm.tn

Keywords
CA19-9, CEA, gender, TNM stages, colorectal cancer, *KCNB1*, personalized medicine, treatment response

Date received: 22 January 2020; accepted: 14 April 2020

Highlights

- Female patients were less frequent to achieve successful treatment outcome.
- Indel rs11468831 is associated with successful colorectal cancer therapy regardless of TNM stages.
- *KCNB1* SNP rs3331 is associated with treatment sustained response.
- CEA and CA19-9 level decline is correlated with CC genotype of rs3331.
- CA19-9 rate decline is correlated with GG genotype of rs1051295.
- *KCNB1* haplotype is not associated with treatment response.

Introduction

Colorectal cancer (CRC) is a major cause of mortality. It is classified as the third leading cause of cancer-related deaths worldwide.[1,2] According to GLOBOCAN's estimations, the incidence of CRC is progressively increasing during the recent decades.[3]

In Tunisia, the age standardized prevalence of CRC is estimated at 6.4/100,000 in 1994, and is expected to rise to 39.3/100.000 by 2024, largely due to the absence of effective disease management.[4,5] The etiology of CRC remains elusive and most likely combines environmental and genetics factors.[6]

The decision-making of treatment in Tunisia is based on established clinical oncology guidelines in light of the uniqueness of Tunisian patients.[7,8]

Folfox, which is a chemotherapy regimen made up of folinic acid (FA), fluorouracil (5-FU), and Oxaliplatin remains the mainstay of standard care for CRC treatment in Tunisia. Carcino-embryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) are two tumor markers widely used for screening disease process and evolution.[9–11] However, their role is limited because of their poor sensitivity to specificity.[12–15] This prompted the search for alternative biomarkers, especially in light of the emergence of resistance to therapy.[7]

The clinical heterogeneity of alteration and defective function of an array of genes was shown to influence the disease process. These include *TP53, KRAS*, and *BRAF* genes.[16–18] Recently, voltage-gated potassium (Kv) channels were associated with numerous neoplasia, principally those affecting the digestive tract.[19,20] Kv2.1, a ubiquitous potassium channel subtype, was shown to be expressed in various tumor cells, and to participate in cell proliferation.[21,22] Kv2.1 is encoded by the *KCNB1* gene, is located on chromosome 20q13.2, and is differentially expressed in several tissues.[23]

Several *KCNB1* variants were implicated in modulating its gene expression.

In spite of the demonstrated association of *KCNB1* with cancer, no study has yet investigated the effect of *KCNB1* polymorphism on CRC progression, or the response to treatment.

In the present study, we examined the association of the *KCNB1* polymorphisms rs3331, rs1051295, and indel related variants rs11468831 on CRC treatment response in Tunisian patients.

Materials and methods

Patient data collection

We performed a longitudinal prospective study that involved 291 CRC patients (family, fCRC and sporadic, sCRC). All cases were followed up in the Salah Azaiz Oncology Institute from February 2016 to January 2018.

The disease stage was assessed according to the TNM classification of the UICC.[24] While the treatment responses are correlated to TNM stage's patients, we stratified the cohort into two groups. The first group (G1) includes patients in stages II and III divided into relapsed and non-relapsed patients and the second group (G2) includes responder and non-responder (stage IV) patients.

Data were collected retrospectively from medical records and personal interviews. Dukes' classification, modified by Astler–Coller, was used to determine CRC stages.[25,26] Patients were followed up after completion of their chemotherapy every 6 months over 3 years, with blood analysis including CBC, CEA, and CA 19-9 levels.

Treatment strategies

The type of the applied treatment depends on the pathological reports and TNM stage. In fact, patients carriers of colon (CC) and rectal cancer (RC) diagnosed with primitive tumor (stage I), undergo surgery directly without being treated with chemotherapy. However, patients diagnosed in stage II with high risk factor were differently treated according to tumor localization. Indeed, CC patients follow an adjuvant

chemotherapy based on fluorouracil (5-FU) and RC patients could undergo neoadjuvant concurrent chemo/radiotherapy as preoperative treatment followed by surgical resection.

All patients with RC and CC in stage III receive postoperative 5-FU-based chemotherapy. Finally, CRC patients with metastatic disease (stage VI) should have first-line chemotherapy according to clinician's medical decision. They are classified as responder and non-responders according to their treatment responsiveness. All patients have undergone surgery according to NCCN (National Comprehensive Cancer Network) and ESMO (European Society for Medical Oncology) Guidelines.

Genotyping assays

Peripheral venous blood was collected in ethylenediaminetetraaceticacid (EDTA)-containing tubes. Genomic DNA was prepared using QIAamp® DNA blood Mini Kit. Genotyping of rs3331, rs1051295 was done by amplification-refractory mutation system-polymerase chain reaction (ARMS-PCR), and standard PCR was used for genotyping rs11468831 variant. Only rs11468831 PCR products were separated using capillary electrophoresis on DNA 500 and DNA 1000 Labchips (Agilent 2100 Bio-analyzer, Biotech Vertriebsgesellschaft m.b.H Agilent Technologies, Lithuania, landlocked). While both rs3331 and rs151295 were confirmed by Sanger sequencing ABI PRISM 3100 DNA Analyzer (Applied Biosystems). Results were analyzed via Sequencer 3.1.1 software.

Radioimmunoassay of CEA and CA 19-9 serum tumor markers

Serum concentration and kinetics follow-up of CEA and CA19-9 tumor markers were assessed by radioimmunoassay (Beckman Coulter, Brea-Calif).[27]

Statistical analysis

Statistical analysis was performed with R for Windows. Continuous variables were expressed as mean ± SD, while categorical data were expressed as percent of the total. Independent sample t-test was used for inter-group comparisons of continuous data which were normally distributed, and chi-square test for categorical variables.

Logistic regression was used in analyzing the independent contribution of key covariates with sustained treatment response; $p < 0.05$ was considered statistically significant. Exhaustive regression was conducted to define the genotype with the real influence on treatments response.

Power of the study was calculated using the following parameters: number of subjects, genotypic relative risk for heterozygous and homozygous minor allele, with assuming the CRC prevalence in Tunisia. Accordingly, the overall power was 88%.

Results

Patients characteristics

The characteristics of patients included in the study are presented in Table 1. No significant differences were observed between groups in terms of age, body mass index (BMI), smoking history, alcohol consumption, hypertension, anemia, extent of tumor differentiation, and histology. On the other hand, among G1 group, the sex-ratio (SR) was significantly different between relapsed (1.34) and non-relapsed patients (0.81) ($p = 0.05$, OR = 0.60, 95% CI = [0.35–0.93]. Weight was significantly lower in non-relapsed patients ($p = 0.0035$) and tumor localization was significantly different between both ($p = 0.049$). Indeed, CC patients (68.5%) have better chances to cancer clearance compared to RC patients (31.55%). Patients in stage II with non-relapsed response are statistically higher (55.05%) and tumor size found as indicator factor to positive response among G1 group ($p = 0.036$). Regarding G2 groups, no differences were detected on the previously mentioned parameters.

Clinical responses to therapy

Stratification factors were essential components of treatment response (i.e. type of treatment and TNM stage). Primary neoadjuvant with Folfox (5-FU + FA) was well established treatment with a high frequency of positive response (non-relapsed) among patients with TNM stage II. In fact, 66.7% of non-relapsed patients were under Folfox treatment versus 33.4% of relapsed cases ($p = 0.0126$). However, 40.81% only of patients belonging in G2 group were responders and 59.19% were non-responders ($p = 0.618$).

Targeting the epidermal growth factor receptor (EGFR) whether alone or in combination with chemotherapy in patients with CRC, had revolutionized the treatment.

In our cohort, 5.15% of patients were under Bevacizumab, among them 40% were non-responder and 33.34% were relapsed. We reported that only 20% of patients achieve a positive response (responders) and 6.66% non-relapsed ($p = 0.0487$ and $p = 0.287$ among G1 and G2, respectively). In addition, 2.40% of total patients received Cetuximab treatment. We found 66.7% of patients were negative responders and only 33.4% had successfully responded to the treatment.

Table 1. Clinicopathological characteristics of the CRC patients.

Parameters	Group 1 (G1)				Group 2 (G2)				p^3
	Non-relapsed N = 149(%)	Relapsed N = 89 (%)	Total N = 238 (%)	p^1	Responder N = 23 (%)	Non-responder N = 30 (%)	Total N = 53 (%)	p^2	
Gender (male/female)	67/82	51/38	118/120	**0.056**	9/14	14/16	23/30	0.515	0.120
Age (years)[2]	59.20 ± 1.00	60.32 ± 1.53	59.62 ± 0.85	0.524	62.04 ± 2.98	62.5 ± 2.17	62.30 ± 1.76	0.795	0.41
Weight (kg)	63.04 ± 13.02	69.40 ± 16.68	65.35 ± 14.70	**0.0035**	70.71 ± 18.91	71.61 ± 16.60	71.25 ± 17.39	0.854	0.85
BMI[2]	24.56 ± 0.38	25.25 ± 0.61	24.28 ± 0.33	0.443	26.94 ± 1.49	26.14 ± 0.90	26.47 ± 0.80	0.609	0.37
Family history of cancer									
sCRC	92(61.74)	51(57.30)	143(60.09)	0.498	10(43.47)	12(40.00)	22(41.51)	0.916	0.626
fCRC	57(38.26)	38(42.69)	95(39.91)		13(56.53)	18(60.00)	31(58.49)		
Smoking	50(33.55)	34(38.20)	84(35.29)	0.468	10(43.47)	12(40.01)	22(41.50)	0.515	0.46
Alcohol	41(27.51)	27(30.33)	68(28.57)	0.641	7(30.43)	7(23.33)	14(26.41)	0.474	0.82
Hypertension	111(74.50)	74(83.14)	185(77.73)	0.123	17(73.91)	18(60.00)	35(60.03)	0.570	0.65
Anemia	36(24.16)	23(25.48)	59(24.68)	0.771	5(21.73)	8(26.66)	13(24.52)	0.325	0.21
Tumor localization									
Colon	102(68.45)	52(58.42)	154(64.70)	**0.049**[a]	16(69.56)	16(53.33)	32(60.37)	0.984	**0.044**
Rectum	47(31.55)	37(41.58)	84(35.30)		7(30.34)	14(46.67)	21(39.62)		
Differentiation									
Well	79(54.10)	48(56.47)	127(54.97)	–	11(47.82)	16(53.33)	27(50.94)	–	–
Moderate	55(37.69)	30(35.29)	85(36.79)	0.711	9(39.13)	13(43.33)	22(41.52)	0.995	0.765
Poor	12(8.21)	7(8.24)	19(8.24)	0.936	3(13.05)	1(3.34)	4(7.54)	0.998	0.511
Histological type (adenocarcinoma)									
Lieberkuhnion	119(84.40)	71(83.53)	190(84.07)	0.610	20(86.95)	24(88.88)	44(88.00)	–	–
Tubular	7(4.98)	6(7.06)	13(5.75)	0.893	1(4.36)	1(3.70)	2(4.00)	0.847	0.904
Mucinous	13(9.21)	6(7.06)	19(8.40)	0.488	2(8.69)	2(7.42)	4(8.00)	0.998	0.571
Signet Ring Cell	2(1.41)	2(2.35)	4(1.78)	0.554	0(0.00)	0(0.00)	0(0.00)	0.995	0.904
TNM classification[b]									
II	72(48.33)	49(55.05)	121(50.85)	**0.047**[a]	–	–	–	–	**0.0414**
III	*77(51.67)*	*40(44.95)*	*117(49.15)*		–	–	–	–	
IV	–	–	–	–	23(100.00)	30(100.00)	53(100.00)		
Tumor size (cm)[2]	11.58 ± 1.57	12.64 ± 2.57	12.25 ± 1.72	**0.036**[a]	8.52 ± 0.98	10.54 ± 1.95	9.95 ± 1.17	0.631	0.84

CRC: colorectal cancer; BMI: body mass index; sCRC: sporadic CRC; fCRC: familial CRC; TNM: tumor, nodes, metastases according to Dukes' Classification modified by Astler–Coller.

[1]Pearson chi-square (categorical variables), Student's t-test (continuous variables).

[2]Mean ± standard deviation.

p^1: relapsed versus non-relapsed.

p^2: responder versus non-responder.

p^3: total population with positive response (non-relapsed + responder) versus total population with negative response (relapsed + non-responder).

Values marked in bold are inferior to 0.05.

[a]Adjusted according to gender.

[b]p^1 calculated by comparing TNM I versus TNM II; p^2 was calculated by comparing TNM III versus TNM VI; p^3 was calculated comparing TNM I, II, III, and VI, and only the associated p of TNM VI was written.

KCNB1 polymorphisms and treatment response among fCRC and sCRC patients

Results of the association between rs3331, rs1051295, and rs11468831 variants with the treatment response are summarized in Table 2.

Most fCRC and sCRC patients carrying rs11468831 homozygous major allele genotype achieved positive response to treatment as non-relapsed ($p = 2.72 \times 10^{-3}$) and/or as responder cases ($p = 0.021$), compared to non-Ins/Ins genotype carriers. In fact, homozygous minor allele genotype frequency was 44.9% among relapsed patients and 38.7% among non-responders. This association remained significant after adjustment for tumor localization, gender, tumor size, and body weight.

The association of rs1051295 genotype with treatment response was comparable in both groups (G1 and G2) ($p = 0.79$ and $p = 0.63$), respectively. However, our analysis reveals an association between rs3331 genotypes and both groups (G1 and G2). Indeed, the association was marginally noticed among G1 patients. Unlikely, the major genotype of rs3331 was not consistently associated with positive treatment response in G1 and G2. Regarding G1 strata, carriage of the homozygous Major rs3331 allele (T/T) was marginally associated with sustained treatment ($p = 0.07$).

Interestingly, we found high association of rs3331 with therapy response G2. In fact, responder patients were frequently carriers of non-homozygous major allele (87%). Moreover, patients carrier of the major homozygous allele (T/T) were 4 folds (3.66) more likely for treatment failing during the therapy process ($p = 0.03$). Consequently, carrying the minor rs3331 allele regardless of the number of copies appears to enhance the positive response for treatment among patients with advanced TNM stages (IV), (Table 2). *KCNB1* haplotypes for rs3331 and rs1051295 did not reveal any association with treatment response (data not shown)

Association between CEA and CA19-9 with KCNB1 polymorphisms and indel mutation and treatment response according to tumor localization

Subsequently, we evaluated the kinetics of decline in CEA and CA19-9 serum levels among CRC patients over the 39 months of treatment period according to the presence of rs3331, rs1051295, rs11468831 (Figure 1), and tumor site (Figure 2).

The magnitude of the decline varied according to the presence of specific variants-genotype. The minor allele rs3331 ($p = 0.043$) and rs1051295 ($p = 0.065$) are associated with pronounced decline in CEA ($p = 0.014$) and CA19-9 ($p = 0.016$). There was no correlation between rs11468831 genotypes and changes in CEA and CA19-9 serum levels during treatment or follow-up periods.

Analysis of CC and RC subgroups revealed associations among RC patients (Fig. 2). Significant differences were also detected between the CEA decline and rs3331 ($p = 0.017$), rs1051295 ($p = 0.006$), and rs11468831 ($p = 0.023$). Likewise, there was a decline in CA19-9 level in rs3331 ($p = 0.029$), rs1051295 ($p = 0.0061$), and rs11468831 ($p = 0.028$) genotype carriers.

Association of KCNB1 polymorphisms and the type of treatment

This identifies spurious factors via a cross-model chi-square statistic that tests for stability in parameter estimates across models. The results from Table 2 confirmed the association of rs3331 and rs1051295 homozygous minor allele genotypes with positive response when patients were treated with 5-FU-based chemotherapy (Folfox) ($p < 0.001$), and to a lesser extent when the Irinitecan is used instead Oxaloplatin (Folfri) ($p < 0.01$). This confirmed the association of the minor genotype of rs3331 and rs1051295 with positive response among patients treated with Folfox ($p < 0.01$) and Folfiri ($p < 0.05$).

Discussion

Despite the availability of therapeutic regimens for CRC treatment, a large proportion of patients do not adequately respond, and thus they are considered as non-responders and/or relapsed. The standard treatment for CRC in Tunisia is financially demanding, leading to the necessity of assessing predictors of response before initiating and during treatment. We, therefore, evaluated the utility of *KCNB1* variants and related polymorphisms as predictors of the treatment response in CRC patients. To the best of our knowledge, this is the first study to examine this aspect of *KCNB1* variants in CRC treatment and follow-up.

SR was different among the relapsed and non-relapsed subgroups 1.34 and 0.81, respectively. Indeed, it is well established that women have a higher risk of developing right-sided (proximal) RC, which is associated with a more aggressive form of neoplasia compared to left-sided (distal) RC. Although differences were revealed in tumor location between women and men[28,29] and the sex-distinguishable results of the most common cancer screening test (iFOBT),[30] colorectal cancer screening guidelines do not distinguish females from males. This may explain the higher observed frequency of more advanced neoplasia when tumors are first detected and false negative results in colonoscopy among females. In addition, the sex-specific response

Table 2. *KCNB1* rs3331, rs1051295, and rs11468831 genotype frequencies according to treatment response.

Polymorphisms	Group 1 (G1), $n = 238$					Group 2 (G2), $n = 53$					Total population		
	Non-relapsed[1]	Relapsed[1]	p^2	OR	CI (95%)	Responder[1]	Non-responder[1]	p^2	OR	CI (95%)	p^3	OR	CI (95%)
rs3331													
T/T	32(29.9)	13(19.4)	0.123	1.00	Ref	3(13.0)	10(32.3)	**0.030**	1.00	Ref	0.695	1.00	Ref
T/C	64 (59.8)	43(64.2)		0.55	0.25-1.22	13(56.5)	18(58.1)		2.79	0.60-13.08		0.89	0.49-1.64
C/C	11(10.3)	11(16.4)		0.33	0.10-1.02	7(30.4)	3(9.7)		13.16	1.60-19.24		1.21	0.54-2.72
T/T	32(29.9)	13(19.4)	**0.07**	1.00	Ref	3(13.0)	10(32.3)	**0.06**	1.00	Ref	0.66	1.00	Ref
T/C-C/C	75 (70.1)	54(80.6)		**0.51**	**0.24-0.91**	20(87.0)	21(67.7)		**3.66**	**0.98-16.20**		1.07	0.56-2.03
rs1051295													
G/G	20(16.5)	13(18.1)	0.964	1.00	Ref	4(17.4)	7(22.6)	0.275	1.00	Ref	0.25	1.00	Ref
A/G	81(66.9)	47(65.3)		1.12	0.50-2.50	18(78.3)	19(61.3)		1.66	0.41-6.64		1.66	0.84-3.26
A/A	20(16.5)	12(16.7)		1.09	0.39-3.06	1(4.3)	5(16.1)		0.35	0.03-4.15		1.13	0.47-2.68
G/G	20(16.5)	13(18.1)	0.79	1.00	Ref	4(17.4)	7(22.6)	0.63	1.00	Ref	0.44	1.00	Ref
A/G-A/A	101(83.5)	59(81.9)		1.11	0.51-2.45	19(82.6)	24(77.4)		1.39	0.35-5.44		1.26	0.70-2.25
rs11468831													
ins/ins	80(53.7)	29(32.6)	$\mathbf{2.75\times10^{-4}}$	1.00	Ref	14(60.9)	9(29.0)	**0.047**	1.00	Ref	$\mathbf{1.4\times10^{-4}}$	1.00	Ref
ins/del	39(26.2)	20(22.5)		**0.75**	**0.39-0.95**	6(26.1)	10(32.3)		**0.35**	**0.09-0.96**		**0.60**	**0.33-1.09**
del/del	30(20.1)	40(44.9)		**0.28**	**0.15-0.53**	3(13.0)	12(38.7)		**0.16**	**0.03-0.80**		**0.25**	**0.14-0.44**
Ins/ins	80(53.7)	29(32.6)	$\mathbf{2.72\times10^{-3}}$	1.00	Ref	14(60.9)	9(29.0)	**0.021**	1.00	Ref	$\mathbf{0.37\times10^{-4}}$	1.00	Ref
Ins/ins-del/del	69(46.3)	60(67.4)		**0.43**	**0.25-0.76**	9(39.1)	22(71.0)		**0.25**	**0.08-0.84**		**0.31**	**0.15-0.68**

OR: odds ratio; CI: confidence interval' CRC: colorectal cancer.

Bold indicates statistical significance ($p \leqslant 0.05$).

[1]Number of subjects (frequencies: percent total).

p^2 value adjusted according to the clinic-pathological parameters of the CRC patients.

p^3 value calculated by comparing total patients with positive response to treatment (non-relapsed + responder) versus total patients with negative response to treatment (relapsed + non responder).

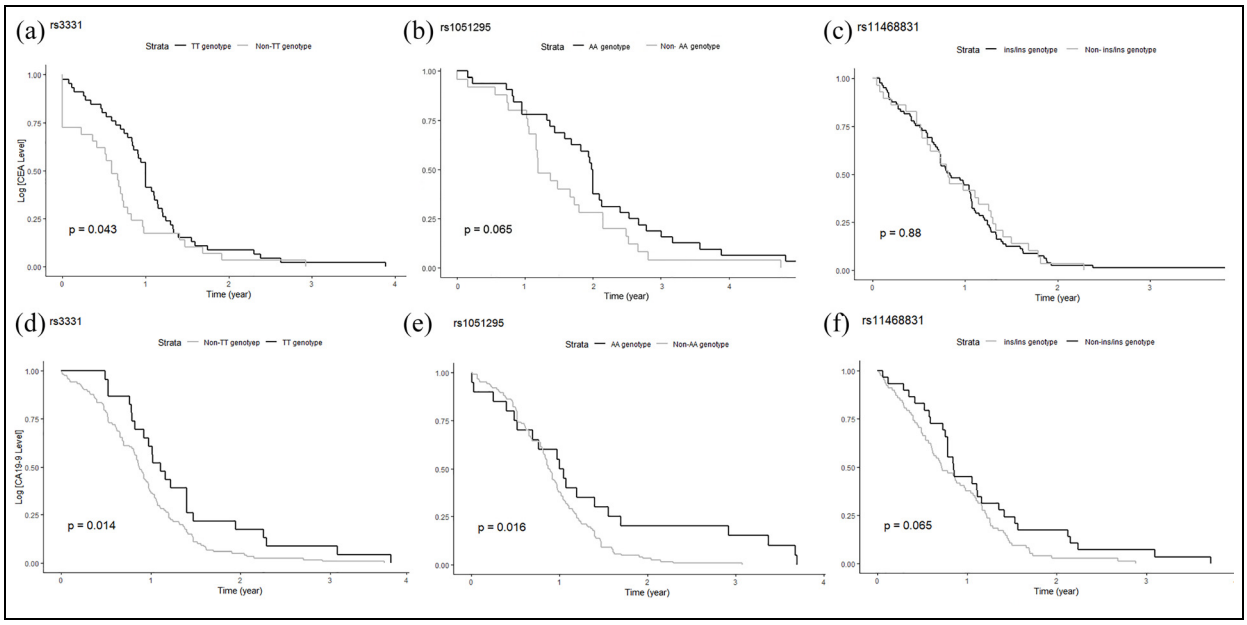

Figure 1. Kinetics of changes in CEA and CA19-9 serum markers among non-responder versus responder CRC patients, according to *KCNB1* genotype status. Polymorphisms. (A, D) rs3331; (B, E) rs1051295; (C, F) rs11468831.

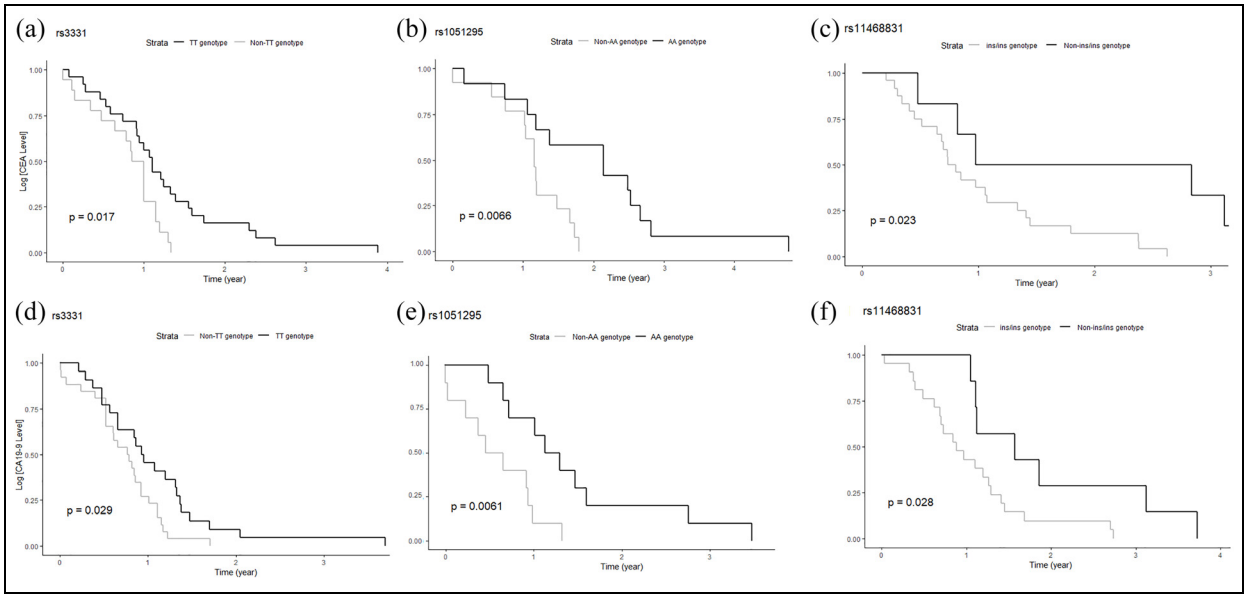

Figure 2. Kinetics of changes in CEA and CA19-9 serum markers among non-responder versus responder RC patients, according to *KCNB1* genotype status. Polymorphisms. A, D) rs3331; (B, E) rs1051295; (C, F) rs11468831.

could be explained by the anticancer drug used, which can cause toxicity to the reproductive system and does not consider the sex effect. Furthermore, the apparent difference in SR between relapsed and non-relapsed might be, to an extent, due to the socioeconomic barriers in Tunisia within female patients that cause delay screening and diagnosis.

We observed significant differences between non-relapsed and relapsed patients in terms of weight, tumor localization, tumor size, and TNM classification. In fact, results of over 7 million individuals, and more than 93,000 patients indicate that weight and/or BMI of 25.0–27.4 kg/m^2, 27.5–29.9 kg/m^2, and >30.0 kg/m^2 were associated with 19%, 24%, and 41% increased risks of developing CRC, respectively, when compared to normal BMI ($\leqslant$ 24 kg/m^2).[31] The non-relapsed had lower mean weight (63.04 kg) and BMI (24.5 kg/m^2) when compared with relapsed (69.40 kg and 24.28 kg/m^2

respectively; $p = 0.0035$). Moreover, a significant association between treatment response and TNM stage was detected.

Indeed, among G1 group, most patients with primary TNM stage fulfill positive results since 5-FU-based chemotherapy improves local control of the tumor. There was a systematic effect of diagnostic TNM stage on responsiveness to chemotherapy; patients with TNM stage IV were less likely to respond to the treatment (64.27%), ($p = 0.041$). Henceforth, the poorer response of patients in colon cancer is likely to be related to delayed diagnosis. Thus, it is possible that more aggressive treatment and/or dose play a role in better response and clearance among these cases. Another puzzling possibility is that our patients with advanced TNM are the oldest subgroups (62.59 ± 12.24 years) compared to others (first, second, and third stages) 49 ± 0.01, 59.25 ± 14.02, and 59.77 ± 12.35 years old, respectively, which can limit the treatment outcomes. In fact, the metastatic tumor cells of young patients may be more sensitive to therapy compared to older patients.[32–34]

In addition to environmental factors, cancer pathogenesis is attributed to mutations in oncogenes or tumor suppressor genes involved in cell division and/or cell death. Increasing evidence demonstrated that ion channel genes contribute to the progression of various carcinomas, and the proliferation of several cell types.[35–40] We explored the correlation between rs3331, rs1051295, and rs11468831 variants and the response to chemotherapy among CRC patients.

By stratifying the total cohort according to tumor localization, our results endorse that most RC patients with "Ins/Ins" genotype were treatment responsive unlike "Del/Del" genotype carriers, who were generally non-responders ($p < 0.001$).

Approximately 45.79% of total CRC patients were carriers of rs11468831 major allele homozygous, and 73.39% of them achieved a positive response to treatment as relapsed while 60.86% was responders from the G2 group.

Roughly 71.2% of patient carriers of rs3331 Major genotype responded positively to the 5 FU-based chemotherapy treatment. It was unlikely that a significant association was revealed among responder and non-responder patients of G2. Indeed, 76.9% of non-responders were carriers of T/T genotype and 70% of responders were patients with minor rs3331 genotype C/C. Consequently, patient carriers of T/T genotype were 3.6-fold more at risk to not fulfilling positive treatment outcomes.

Hence, we hypothesize that both the rs3331C/C genotype and rs11468831 ins/ins genotype constitute favorable genotypes to improve the treatment response.

It is possible that the carriage of these variants could alter the ion channel normal function, causing impairment in pathways involved in tumor exclusion and treatment response. Recently, it was suggested that *KCNB1*-induced autophagy inhibits tumor growth, and increases CRC survival,[41] and that autophagy represents a key point in CRC aggressiveness and progression.[39,42–46] Indeed, It is well established that cancer cells can overcome autophagy and survive by avoiding the stress of anticancer drugs.[47,48] The cause–effect nature of the association between variants and response to treatment in CRC remains to be understood. Our results and growing body of evidence linking *KCNB1*, autophagy, cancer progression, and treatment response prompts the speculation that these variants may constitute a promising determinant of the chemotherapy outcomes. Currently, the widely used diagnostic procedure for CRC is endoscopy, which is highly sensitive and specific in identifying CRC.[12,49,50] Yet, the high cost, invasive nature, and need for repeated testing resulted in limited implementation of endoscopy for CRC follow-up. Consequently, blood biomarkers constitute efficient indicators for CRC evaluation, and monitoring the treatment response, which are mainly CEA and C19-9.[51] However, it lacks the specificity, as it is associated with other cancer types.[52]

Due to the heterogeneity of CRC phenotypes, a single tumor marker, such as CA19-9, is not considered a stand-alone diagnostic test. Significant associations were found between rs3331 genotypes and kinetics of CEA and CA19-9 among responder patients with the minor genotype being favorable for the decline in CEA and CA19-9 levels, and thus treatment response. Yet, no clear association was seen between rs1051295 genotypes and CEA kinetics among responder patients. Moreover, carriage of Ins/Ins genotype was correlated to a faster decline in CEA and CA19.9 levels, when compared to Del/Del genotype.

Our results recommend gender-specific strategy for screening and treatment. Also, prevention protocols can be established to reduce the treatment failure and improve the quality of life. In addition, as these polymorphisms may modulate treatment response and chemotherapy pathway, its screening, before starting the chemotherapy session may enhance the positive responsiveness.

These results sustain the rational for considering new approaches for safe and effective chemotherapy, among Tunisian CRC patients. Moreover, these polymorphisms appear to be reliable markers for monitoring the disease kinetics, and treatment response, compared to CEA and CA19-9. In fact, in spite of CEA and/or CA19-9 levels are often elevated among patients with gastrointestinal malignancies, patients with conformed cancers frequently have normal levels similar to healthy subjects.[53] In addition, elevated CEA levels may be detected in smokers as well as patients with a variety of non-malignant diseases. Hence, proposing new genetics

tools could be effective modalities to surrogate tumor markers and manage, effectively, the asymptomatic individuals for CEA and CA19-9.

The limits of this study are related to the retrospective side. In addition, not all patients have been able to use EGFR target therapy, due to the public healthcare system that does not cover the treatment charges. Nevertheless, this study could serve to extend our understanding of the phenotype aspects of CRC in Tunisia.

Conclusion

The assessment of CRC patients to chemotherapy is crucial. Our results showed that women are more prone to the aggressive form of CRC. Moreover, our analysis supports the notion that screening of the *KCNB1* gene and related variants may improve the effectiveness of treatment by implementation of personalized approach for patients with unfavorable genotype in order to monitor the disease in a better way.

Acknowledgements

The authors express their thanks to Drs Hechmi Louzir and Riadh Kharrat for their encouragement. They also thank Drs Alia Benkahla and Mouna Stayoussef for excellent assistance with experiments and discussions.

Authors' contributions

MB carried out the experiments and manuscript preparation, IS conducted statistical analysis and manuscript drafting, SB carried out sample processing, RBA assisted with molecular data acquisition, HK performed bioanalyzer data acquisition, AF did sample processing, RH studied the concept of the original idea, AMO dealt with patient recruitment, AME supervised clinical data acquisition, WAY discussed the results and contributed to the final manuscript, BYW contributed to the final manuscript, and BBZ studied the concept and design, data interpretation, and final manuscript preparation. All authors read and approved the final manuscript.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on request.

Consent for publication

All authors have carefully examined and approved the content of the manuscript, and understand and accept that in the event of its publication, all copyright shall be transferred to the *Tumor biology* journal.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical approval

All the patient investigations conformed to the principles outlined in the Declaration of Helsinki and have been performed with the permission of Oncology Department released by the Ethics Committee of Salah Azaiz Oncology Institute (SAI) (Registration No.: ISA/2016/02. All the patients were informed about the purpose of the study, and consented in writing to participate in the study.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was partially supported by funding from the Institute Pasteur of Tunis, the Ministry of High Education and Scientific Research (MESRS) with grants from the International Foundation for Science (F2762-2), NATO project (SFP981865) and H3ABioNet network.

ORCID iDs

Ikram Sghaier https://orcid.org/0000-0001-8198-7668
Wassim Y Almawi https://orcid.org/0000-0003-1633-9757
Balkiss Bouhaouala-Zahar https://orcid.org/0000-0003-3147-245X

References

1. Siegel RL, Miller KD and Jemal A. Cancer statistics. *CA Cancer J Clin* 2019; 69: 7–34.
2. Smith RA, Manassaram-Baptiste D, Brooks D, et al. Cancer screening in the United States, 2015: a review of current American Cancer Society guidelines and current issues in cancer screening. *CA Cancer J Clin* 2015; 65: 30–54.
3. Cronin KA, Lake AJ, Scott S, et al. Annual report to the nation on the status of cancer, part I: national cancer statistics. *Cancer* 2018; 124: 2785–2800.
4. Arfaoui TA, Ben Mahmoud LK, Khiari M, et al. Etude epidemiologique, anatomopathologique et evaluation des facteurs pronostiques des adénocarcinomes colorectaux mucineux vc non mucineux. (A propos d'une serie de 196 patients). *La Tunis Méd* 2010; 88(1): 12–17.
5. Khiari H, Ben Ayoub HW, Ben Khadhra H, et al. Colorectal cancer incidence trend and projections in Tunisia (1994-2024). *Asian Pac J Cancer Prev* 2017; 18(10): 2733–2739.
6. Sethi G, Shanmugam MK, Ramachandran L, et al. Multifaceted link between cancer and inflammation. *Biosci Rep* 2012; 32(1): 1–15.
7. Hammond WA, Swaika A and Mody K. Pharmacologic resistance in colorectal cancer: a review. *Ther Adv Med Oncol* 2016; 8(1): 57–84.
8. Treatment of colon cancer, by stage, https://www.cancer.org/cancer/colon-rectal-cancer/treating/by-stage-colon.html (accessed 30 July 2018).
9. Sikorska H, Shuster J and Gold P. Clinical applications of carcinoembryonic antigen. *Cancer Detect Prev* 1988; 12: 321–355.
10. Goldberg EM, Simunovic LM, Drake SL, et al. Comparison of serum CA 19-9 and CEA levels in a population at

high risk for colorectal cancer. *Hybridoma* 1989; 8(5): 569–575.
11. Zhai H, Huang J, Yang C, et al. Serum CEA and CA19-9 levels are associated with the presence and severity of colorectal neoplasia. *Clin Lab* 2018; 64(3): 351–356.
12. Gao Y, Wang J, Zhou Y, et al. Evaluation of serum CEA, CA19-9, CA72-4, CA125 and ferritin as diagnostic markers and factors of clinical parameters for colorectal cancer. *Sci Rep* 2018; 8(1): 2732.
13. Locker GY, Hamilton S, Harris J, et al. ASCO 2006 update of recommendations for the use of tumor markers in gastrointestinal cancer. *J Clin Oncol* 2006; 24(33): 5313–5327.
14. Sturgeon CM, Hoffman BR, Chan DW, et al. National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines for use of tumor markers in clinical practice: quality requirements. *Clin Chem* 2008; 54(8): e1–e10.
15. Tomasevic R, Milosavljevic T, Stojanovic D, et al. Predictive value of carcinoembryonic and carbohydrate antigen 19-9 related to some clinical, endoscopic and histological colorectal cancer characteristics. *J Med Biochem* 2016; 35(3): 324–332.
16. Soussi T and Beroud C. Assessing TP53 status in human tumours to evaluate clinical outcome. *Nat Rev Cancer* 2001; 1(3): 233–240.
17. Schubbert S, Shannon K and Bollag G. Hyperactive ras in developmental disorders and cancer. *Nat Rev Cancer* 2007; 7(4): 295–308.
18. Wan PTC, Garnett MJ, Roe SM, et al. Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. *Cell* 2004; 116(6): 855–867.
19. Chin LS, Park CC, Zitnay KM, et al. 4-aminopyridine causes apoptosis and blocks an outward rectifier K+ channel in malignant astrocytoma cell lines. *J Neurosci Res* 1997; 48(2): 122–127.
20. Woodfork KA, Wonderlin WF, Peterson VA, et al. Inhibition of ATP-sensitive potassium channels causes reversible cell-cycle arrest of human breast cancer cells in tissue culture. *J Cell Physiol* 1995; 162(2): 163–171.
21. Li M and Xiong Z-G. Ion channels as targets for cancer therapy. *Int J Physiol Pathophysiol Pharmacol* 2011; 3(2): 156–166.
22. Pancrazio JJ, Tabbara IA and Kim YI. Voltage-activated K + conductance and cell proliferation in small-cell lung cancer. *Anticancer Res* 1993; 13(4): 1231–1234.
23. Suzuki T and Takimoto K. Selective expression of HERG and Kv2 channels influences proliferation of uterine cancer cells. *Int J Oncol* 2004; 25(1): 153–159.
24. Van Mater D and Wagner L. Management of recurrent Ewing sarcoma: challenges and approaches. *Onco Targets Ther* 2019; 12: 2279–2288.
25. Dukes CE. The classification of cancer of the rectum. *J Pathol Bacter* 1932; 35: 323–332.
26. Astler VB and Coller FA. The prognostic significance of direct extension of carcinoma of the colon and rectum. *Ann Surg* 1954; 139(6): 846–852.
27. Sato T, Nishimura G, Nonomura A, et al. Serological studies on CEA, CA 19-9, sTn and SLX in colorectal cancer. *Hepatogastroenterology* 1998; 46(26): 914–919.
28. Hansen IO and Jess P. Possible better long—term survival in left versus right—sided colon cancer —a systematic review. *Dan Med J* 2012; 59(6): A4444.
29. Pal SK and Hurria A. Impact of age, sex, and comorbidity on cancer therapy and disease progression. *J Clin Oncol* 2010; 28(26): 4086–4093.
30. Brenner H, Haug U and Hundt S. Sex differences in performance of fecal occult blood testing. *Am J Gastroenterol* 2010; 105(11): 2457–2464.
31. Meyerhardt JA, Kroenke CH, Prado CM, et al. Association of weight change after colorectal cancer diagnosis and outcomes in the Kaiser Permanente Northern California population. *Cancer Epidemiol Biomarkers Prev* 2017; 26(1): 30–37.
32. Walker AS, Zwintscher NP, Johnson EK, et al. Future directions for monitoring treatment response in colorectal cancer. *J Cancer* 2014; 5(1): 44–57.
33. Hemminki K, Santi I, Weires M, et al. Tumor location and patient characteristics of colon and rectal adenocarcinomas in relation to survival and TNM classes. *BMC Cancer* 2010; 10(1): 688.
34. Haque S. Diagnostic significance of CEA and CA 19-9 for the early diagnosis of cancer. *J Med Chem Toxicol* 2019; 4(1): 13.
35. Lan M, Shi Y, Han Z, et al. Expression of delayed rectifier potassium channels and their possible roles in proliferation of human gastric cancer cells. *Cancer Biol Ther* 2005; 4(12): 1342–1347.
36. Thiffault I, Speca DJ, Austin DC, et al. A novel epileptic encephalopathy mutation in KCNB1 disrupts Kv2.1 ion selectivity, expression, and localization. *J Gen Physiol* 2015; 146(5): 399–410.
37. Xin J, Du M, Gu D, et al. Combinations of single nucleotide polymorphisms identified in genome-wide association studies determine risk for colorectal cancer. *Int J Cancer* 2019; 145: 2661–2669.
38. Lang F and Stournaras C. Ion channels in cancer: future perspectives and clinical potential. *Philos Trans R Soc Lond B Biol Sci* 2014; 369(1638): 20130108.
39. Litan A and Langhans SA. Cancer as a channelopathy: ion channels and pumps in tumor development and progression. *Front Cell Neurosci* 2015; 9: 86.
40. Prevarskaya N, Skryma R and Shuba Y. Ion channels in cancer: are cancer hallmarks oncochannelopathies. *Physiol Rev* 2018; 98(2): 559–621.
41. Wang H-Y, Wang W, Liu YW, et al. Role of KCNB1 in the prognosis of gliomas and autophagy modulation. *Sci Rep* 2017; 7(1): 14.
42. Marinkovic M, Sprung M, Buljubasic M, et al. Autophagy modulation in cancer: current knowledge on action and therapy. *Oxid Med Cell Longev* 2018; 2018: 8023821.
43. Li Y-J, Lei Y-H, Yao N, et al. Autophagy and multidrug resistance in cancer. *Chin J Cancer* 2017; 36(1): 52.
44. Qian H-R, Shi Z-Q, Zhu H-P, et al. Interplay between apoptosis and autophagy in colorectal cancer. *Oncotarget* 2017; 8(37): 62759–62768.
45. Koustas E, Sarantis P, Kyriakopoulou G, et al. The interplay of autophagy and tumor microenvironment in colorectal cancer—ways of enhancing immunotherapy action. *Cancers* 2019; 11(4): 533.

46. D'Arcangelo D, Scatozza F, Giampietri C, et al. Ion channel expression in human melanoma samples: in silico identification and experimental validation of molecular targets. *Cancers* 2019; 11(4): 446.
47. Sinha G. Autophagy inhibitors: the hunt is on. *J Natl Cancer Inst* 2016; 108(10): djw245.
48. Garbar C, Mascaux C, Giustiniani J, et al. Chemotherapy treatment induces an increase of autophagy in the luminal breast cancer cell MCF7, but not in the triple-negative MDA-MB231. *Sci Rep* 2017; 7: 7201.
49. Kahi CJ, Boland CR, Dominitz JA, et al. Colonoscopy surveillance after colorectal cancer resection: recommendations of the US multi-society task force on colorectal cancer. *Am J Gastroenterol* 2016; 150: 758–768.
50. Fisher DA, Shergill AK, Early DS, et al. Role of endoscopy in the staging and management of colorectal cancer. *Gastrointest Endosc* 2013; 78(1): 8–12.
51. Abaza H, Ghanem A, Jmal A, et al. Interet des dosages sériques de la proteine c reactve (crp), de l'antigene carcino embryonnaire (ace) et de la lacticodeshydrogenase (ldh) dans le cancer colorectal. *La Tunis Méd* 2010; 88(6): 409–413.
52. Dasgupta A and Wahed A. *Clinical chemistry, immunology and laboratory quality control: a comprehensive review for board preparation, certification and clinical practice*. Cambridge, MA: Academic Press, 2013.
53. Champely S. *pwr: basic functions for power analysis*. R Package Version 1.1. 1. Vienna: The R Foundation, 2009.