View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by FarFar - Repository of the Faculty of Pharmacy, University of Belgrade

EPMA Journal (2019) 10:307–316
https://doi.org/10.1007/s13167-019-00178-x

RESEARCH

Association among resistin, adenylate cyclase-associated protein 1 and high-density lipoprotein cholesterol in patients with colorectal cancer: a multi-marker approach, as a hallmark of innovative predictive, preventive, and personalized medicine

Marija Mihajlovic[1] · Ana Ninic[1] · Miron Sopic[1] · Milica Miljkovic[1] · Aleksandra Stefanovic[1] · Jelena Vekic[1] · Vesna Spasojevic-Kalimanovska[1] · Dejan Zeljkovic[2] · Bratislav Trifunovic[2,3] · Zeljka Stjepanovic[4] · Aleksandra Zeljkovic[1]

Received: 11 March 2019 / Accepted: 10 July 2019 / Published online: 20 July 2019
© European Association for Predictive, Preventive and Personalised Medicine (EPMA) 2019

Abstract

Background Elevated concentrations of resistin have been reported in colorectal cancer (CRC), but its interactions with adenylate cyclase-associated protein 1 (CAP-1) are largely unexplored. We investigated resistin plasma concentration, peripheral blood mononuclear cells (PBMCs) resistin messenger ribonucleic acid (mRNA), and CAP-1 mRNA levels in CRC patients, as well as the impact of resistin gene polymorphism rs1862513 on the examined markers. We also explored associations of resistin with high-density lipoprotein cholesterol (HDL-C) and predictive potential of our parameters for CRC.

Methods Eighty-six patients with CRC and 75 healthy adults were included. Commercial ELISA kit was used for obtaining resistin's concentrations, while polymerase chain reaction (PCR) method was applied for evaluation of resistin and CAP-1 mRNA levels and rs1862513 polymorphism.

Results Plasma resistin and CAP-1 mRNA levels were higher in CRC patients ($p < 0.001$ and $p < 0.05$, respectively), while resistin mRNA levels were lower ($p < 0.001$). Negative association existed among plasma resistin and HDL-C concentrations ($\rho = -0.280$; $p < 0.05$). A model including age, body-mass index, HDL-C, low-density lipoprotein cholesterol (LDL-C), and plasma resistin concentrations as independent predictors of CRC showed very good diagnostic accuracy (AUC = 0.898). We found no associations of rs1862513 with the examined markers.

Conclusions Our study demonstrated increased plasma resistin and CAP-1 mRNA levels, implying their possible interaction in CRC. The association among plasma resistin and HDL-C might indicate that HDL-C is involved in alterations of resistin's secretion process. As a hallmark of personalized medicine, multi-marker approach in determination of resistin-related parameters might be useful for prediction and prevention of CRC development.

Keywords Resistin · Adenylate cyclase-associated protein 1 · High-density lipoprotein cholesterol · Paraoxonase-1 activity · Colorectal cancer · Predictive preventive personalized medicine

✉ Marija Mihajlovic
marijamihajlovic89@yahoo.com

[1] Department of Medical Biochemistry, Faculty of Pharmacy, University of Belgrade, POB 146, Belgrade 11000, Serbia

[2] Clinic of General Surgery, Military Medical Academy, Belgrade, Serbia

[3] Faculty of Medicine of the Military Medical Academy, University of Defence, Belgrade, Serbia

[4] Medigroup General Hospital, Belgrade, Serbia

Introduction

Colorectal cancer (CRC) is a progressive disease, driven by accumulated genetic and epigenetic changes and unhealthy life habits [1]. Inflammation and adiposity are considered as contributing factors in CRC development [2, 3], but mechanisms that associate these metabolic alterations with CRC are still not completely understood. Resistin is one of candidate molecules that might have a significant role in the interplay of inflammation, adiposity, and carcinogenesis. Although resistin is considered as an adypocytokine, a translational

study by Schwartz and Lazar implied that human resistin primarily originates from macrophages, rather than from adipocytes [4]. Increased resistin concentrations are commonly seen in diabetes mellitus, metabolic syndrome, and inflammatory bowel disease [5–7], but previous studies have also shown that elevated resistin levels are associated with the increased risk for development of CRC [8]. Resistin is believed to contribute to cancer expansion by stimulating malignant cell growth and proliferation, but the experiments conducted in order to verify this hypothesis yielded contradictory results [9]. Despite a disputable role of resistin in cell proliferation during tumorigenesis, its relationship with inflammation in malignant diseases is unquestionable [10]. However, it is largely unknown whether resistin, being an adipocytokine, also participates in development of dyslipidemia which is commonly seen in CRC [11]. The relationship of resistin with high-density lipoprotein (HDL) particles might be especially intriguing, since this lipoprotein is a significant contributor to anti-inflammatory and anti-oxidative capacities of plasma. We have previously demonstrated alterations of high-density lipoprotein (HDL) particles maturation, antioxidative capacity [12], and structure [13] in CRC. Nevertheless, possible associations of resistin with HDL cholesterol (HDL-C) concentration and paraoxonase-1 (PON-1) activity, which is an indicator of HDL's protective properties, are still unexplored in this pathological condition. In view of cancer complexity, it was already postulated that only a comprehensive approach, which comprises integrative multi-parameter analysis, can lead to thorough understanding of carcinogenesis and therefore can assure predictive, preventive, and personalized medicine practice [14, 15]. In this context, exploration of interactions among resistin, HDL-C and PON-1, as hallmarks of disturbed inflammatory, lipid, and redox balance, might be useful in defining more accurate panel of biomarkers of increased CRC risk. Consequently, the use of appropriate and integrated cluster of markers could enhance disease prediction, targeted prevention, and personalized treatment.

Given that the significance of resistin was confirmed in various pathological conditions, it has been sought for an adequate receptor through which resistin achieves its effects. In humans, Toll like receptor 4 (TLR4) was considered as a principal target for resistin's binding [16], until Sahmin Lee et al. discovered that adenylate cyclase-associated protein 1 (CAP-1) can serve as a receptor for resistin [17]. The same authors showed that resistin-CAP-1 interaction is highly capable to activate protein kinase A (PKA) and nuclear factor kappa B (NF-kB) signaling pathway, which further leads to inflammatory cytokines production [17]. High blood concentrations of resistin have often been associated with CRC [18], but CAP-1 gene expression was investigated in patients with cardiovascular diseases [19], while resistin-CAP-1 interaction is still unexplored in CRC. Yet, detection of plausible patterns of alterations in protein-receptor complex could be important for prediction of risk, prevention, and personalized treatment of CRC, as for any other malignant disease [20]. Additionally, modern concept of personalized medicine is mostly based on understanding of the associations among genetic and epigenetic alterations and individual clinical outcomes [21]. Being one of the most common sources of genetic diversity, single nucleotide polymorphism (SNP) analysis should not be neglected while deriving final conclusions regarding resistin's role in cancer. Ample of evidences reported the association of resistin gene (*RETN*) -420 C/G polymorphism (rs1862513) with higher CRC incidence [22]. In light of this, it could be of interest to explore the relationship among this particular SNP and resistin protein and messenger ribonucleic acid (mRNA) levels, as well the association with CAP-1 mRNA levels.

The aim of our study was to investigate peripheral blood mononuclear cells (PBMCs) resistin mRNA levels and resistin plasma concentration, as well as PBMC CAP-1 mRNA levels in patients with CRC. Next, we investigated the influence of *RETN* polymorphism rs1862513 on resistin circulating levels and genetic markers. In addition, we explored possible associations of resistin with HDL-C and activity of PON-1.

Subjects and methods

Patients

This research was designed as a part of a larger project that investigated interactive role of dyslipidemia, oxidative stress, and inflammation in CRC, enrolling 126 patients as previously reported [12, 13]. Patients were recruited from the Clinic of General Surgery, Military Medical Academy in Belgrade from July 2014 to January 2016. All eligible patients underwent elective resection for CRC. Patients were enrolled according to the following criteria: adult age, the first onset of CRC, personal anamnesis without any previous malignant diseases, no prior treatment with neoadjuvants, no severe physical disability, and no use of anti-hyperlipidemic drugs. Each patient underwent postoperative histological confirmation of CRC. In 5 cases, postoperative pathohistological findings excluded CRC, while in 121 cases, adenocarcinoma was confirmed. Standardized questionnaires containing basic information on age, height, weight, smoking status, physical activity, personal, and family anamnesis were fulfilled for each patient. Due to incomplete data or uncompleted biochemical and genetic analyses, 40 subjects were excluded, so the final study group consisted of 86 patients with CRC (26 women and 60 men).

The control group included 75 healthy adults, who were recruited during routine medical check-ups at the Medigroup General Hospital in Belgrade. Inclusion criteria were absence of any present or previous malignant disease; absence of

chronic heart, liver, or kidney disease; and no use of any anti-hyperlipidemic medications. All healthy subjects fulfilled the same questionnaire as CRC patients. A signed informed consent was obtained for every participant before the enrolment. All procedures and study design were conducted according to the ethical guidelines defined by the Helsinki Declaration and approved by the local ethical committees.

Laboratory analyses

Blood samples from patients were collected immediately before surgical procedure and from healthy participants at the commencement of a medical examination. Samples were drawn in serum and EDTA plasma collecting vacutainers (BD Vacutainer®, New Jersey, USA), after a 12-h fasting period and 200 μl of whole blood were separately taken into sterilized tubes for SNP analysis. Plasma and serum were separated by centrifugation at 1500×*g* for 10 min and 15 min, respectively, and stored at − 80 °C. The samples were analyzed immediately after thawing. Ficoll-Paque™ PLUS (GE Healthcare, Waukesha, Wisconsin, USA) density gradient medium was used for obtaining PBMCs from EDTA tubes. TRIzol™ (Ambion, Life technologies, Grand Island, New York) reagent for degradation of cells and preservation of total ribonucleic acids (tRNA) at − 80 °C was immediately added to isolated PBMCs. Isolation of tRNA was done according to adjusted protocol of TRIzol™ reagent manufacturer [23]. DNA isolation from whole blood was performed using silica matrix columns according to the manufacturer instructions (GeneJET Whole Blood Genomic DNA Purification Mini Kit; ThermoFisher Scientific).

Gene expression analysis and genotyping were performed on 7500 Real-Time Polymerase Chain Reaction (PCR) System (Applied Biosystems, Foster City, CA, USA). Reverse transcription was achieved using High-Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA). Relative quantification of gene expression was performed using TaqMan™ Gene Expression Assays (Applied Biosystems, Foster City, CA, USA). For determination of human *RETN* levels, we used Hs00220767 m1 assay and for *CAP-1*, Hs00255173 m1 assay was applied, while *beta actin* (Hs99999903 m1 assay) was employed as constitutively expressed gene for gene expression data normalization. The results were derived by the relative standard curve method, while negative controls for reverse transcription, as well as non-template controls, were used for follow-up of reagent's contamination. Third control was transcriptase free control. Data are presented as a ratio of target gene mRNA and beta actin mRNA, namely normalized resistin mRNA = resistin mRNA/beta-actin mRNA; normalized CAP-1 mRNA = CAP-1 mRNA/beta-actin mRNA. Genotyping of rs1862513 (TaqMan® SNP Genotyping Assays) was accomplished according to the instructions of the reagent manufacturer (Applied Biosystems, Foster City, CA, USA).

Routine laboratory assessment included determination of total cholesterol (TC), HDL-C, low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG). All analyses were performed on automated analyzer ILAB 300+ (Instrumentation Laboratory, Milan, Italy), according to the reagent manufacturer protocols (Biosystems SA, Barcelona, Spain). PON-1 activity was measured kinetically by the use of substrate paraoxon. Commercial Elisa test was applied for obtaining resistin plasma concentrations (R&D Systems, Minneapolis, Minnesota).

Statistics

Normality of data was tested by Kolmogorov-Smirnov test. Symmetrically distributed variables are presented as mean ± standard deviation. Parameters that followed normal distribution after logarithmic transformation are presented as geometrical mean with 95% confidence interval. Skewed data are presented as medians with interquartile ranges. We used Mann-Whitney *U* test, Kolmogorov-Smirnov *Z* test, and Kruskal-Wallis test for comparison of non-symmetrically distributed data. Student's *t* test was applied for exploring differences among normally distributed variables and chi-square test for analysis of categorical variables. Spearman's correlation analysis was conducted for evaluating statistically significant associations among the studied parameters. Univariate binary logistic regression analysis was applied for identification of possible risk factors for CRC development. Significant risk factors, singled out by the univariate analysis, were further tested by multivariate binary logistic regression analysis, in order to estimate their independent contribution to increased risk for CRC development. Already known anthropometric and lipid risk factors for CRC were used as confounders in multivariate analysis, including age, gender, BMI, HDL-C, and LDL-C. Receiver operating characteristics (ROC) curves were employed, and the areas under ROC curves (AUC) were calculated for estimation of clinical accuracy of the examined parameters. Separate ROC curves were created for the following parameters: plasma resistin concentration, normalized CAP-1 mRNA, and plasma resistin/resistin mRNA ratio. Further evaluation of diagnostic accuracy was achieved for clusters of examined parameters by formation of three different models. Model 1 included normalized CAP-1 mRNA levels and plasma resistin concentration; in Model 2, we estimated discriminative ability of combined plasma resistin, PON-1 activity, and HDL-C concentration, while in Model 3, all independent significant markers detected by multivariate binary regression analysis were involved: age, BMI, HDL-C, LDL-C, and plasma resistin concentrations. IBM® SPSS® model 22.0

and SNPStats online software (© 2006 Institut Català d'Oncologia) were used for statistical analyses. Differences at $p < 0.05$ were considered as significant.

Results

General anthropometric and laboratory characteristics of study participants are presented in Table 1. The age and gender differences were statistically significant, with CRC patients being older and more prevalent males. Body mass index (BMI) was lower in patients. Evaluation of laboratory parameters singled out lower concentrations of TC, LDL-C, and HDL-C in patient group (Table 1). Significantly higher plasma concentrations of resistin (median 19.692 ng/mL, interquartile range 14.192–28.282 ng/mL vs median 10.537 ng/mL, interquartile range 7.838–17.860 ng/mL; $p < 0.001$), in parallel with lower resistin mRNA levels (median 0.444, interquartile range 0.312–0.643 vs median 0.693, interquartile range 0.508–1.209; $p < 0.001$), were observed in CRC group when compared to controls. Plasma resistin/resistin mRNA ratio was higher in CRC patients (median 42.519, interquartile range 23.514–63.179), than in the control group (median 13.937, interquartile range 10.199–24.492; $p < 0.001$). Higher levels of CAP-1 mRNA were also found in patients (median 0.931, interquartile range 0.789–1.143 vs median 0.862, interquartile range 0.732–1.012; $p < 0.05$). Serum PON-1 activity was higher in the control group (median 284.000 U/L, interquartile range 206.500–580.000 U/L) when compared to CRC patients (median 204.000 U/L, interquartile range 134.000–617.000 U/L), although with borderline statistical significance according to Mann-Whitney U test ($p = 0.069$). However, when a more sensitive Kolmogorov-Smirnov Z test for parameters with high inter-individual variability was conducted, statistically significant difference was achieved for comparison of PON-1 activity in CRC patients and control subjects ($p = 0.017$).

Statistically significant positive correlations of plasma resistin levels with BMI and TG were noticed in the control group (Table 2). On the other hand, in CRC patients, a positive association was found among BMI and CAP-1 mRNA levels, while BMI and HDL-C were in negative correlation with plasma resistin concentrations. In addition, a positive correlation of resistin plasma levels with resistin mRNA was noted in patients, although without reaching statistical significance (Table 2). Since we aimed to further investigate the observed association among resistin protein and mRNA levels, we divided CRC patients into tertiles according to plasma resistin concentration. We observed a rise in resistin mRNA across tertiles of plasma resistin, with statistically significant difference among the second and the third tertile (Fig. 1). Additionally, in order to elucidate previously observed association among HDL-C and plasma resistin concentrations, we divided CRC patients according to HDL-C tertiles. We observed a significant increase in resistin plasma levels in the lowest tertile, when compared to the groups with intermediate and high levels of HDL-C (Fig. 2a). Furthermore, a positive correlation among HDL-C and PON-1 enzyme activity, which existed in CRC patients ($\rho = 0.322$; $p < 0.01$), led us to investigate alterations of PON-1 activity across HDL tertiles (Fig. 2b). PON-1 activity was significantly increased in the highest HDL-C tertile group when compared to the lowest.

We also sought to explore possible independent associations of all examined markers of resistin status with the risk for CRC development. For that purpose, we employed univariate and multivariate binary logistic

Table 1 General anthropometric and laboratory data in CRC patients and control group

Parameter	CRC patients ($N = 86$)	Control group ($N = 75$)	p
Gender (f/m)	26/60	37/38	< 0.050
Age (years)	64.660 ± 10.999	54.670 ± 7.792	< 0.001
BMI (kg/m^2)	25.105 ± 3.126	26.256 ± 3.966	< 0.050
TC (mmol/L)	4.500 ± 1.221	5.702 ± 1.029	< 0.001
HDL-C (mmol/L)	1.059 ± 0.408	1.384 ± 0.539	< 0.001
LDL-C (mmol/L)	2.841 ± 1.088	3.681 ± 0.982	< 0.001
TG (mmol/L)[a]	1.266 (1.176–1.364)	1.303 (1.187–1.430)	0.629
Resistin (ng/mL)[b]	19.692 (14.192–28.282)	10.537 (7.838–17.860)	< 0.001
Normalized CAP-1 mRNA[b]	0.931 (0.789–1.143)	0.862 (0.732–1.012)	< 0.050
Normalized resistin mRNA[b]	0.444 (0.312–0.643)	0.693 (0.508–1.209)	< 0.001
Plasma resistin/resistin mRNA[b]	42.519 (23.514–63.179)	13.937 (10.199–24.492)	< 0.001
PON1 activity (U/L)[b]	204.000 (134.000–617.000)	284.000 (206.500–580.000)	0.069

Data are presented as mean ± standard deviation and compared by Student's t test

[a] Data are presented as geometrical mean (95% confidence interval for mean)

[b] Data are presented as median with interquartile range. Comparison was performed by Mann-Whitney U test

Table 2 Significant correlations of plasma resistin and normalized CAP1 mRNA levels with other examined parameters in CRC patients and control group

Parameter	Resistin (ng/mL)	Normalized CAP-1 mRNA
CRC patients		
BMI (kg/m^2)	ρ = − 0.245; p < 0.050	ρ = 0.222; p = 0.050
HDL-C (mmol/L)	ρ = − 0.280; p < 0.050	$\rho = 0.062$; $p = 0.571$
Normalized resistin mRNA	ρ = 0.204; p = 0.076	$\rho = 0.083$; $p = 0.458$
Control group		
BMI (kg/m^2)	ρ = 0.434; p < 0.001	$\rho = 0.069$; $p = 0.564$
TG (mmol/L)	ρ = 0.277; p < 0.050	$\rho = 0.003$; $p = 0.977$
Normalized resistin mRNA	$\rho = 0.078$; $p = 0.521$	$\rho = 0.132$; $p = 0.259$

ρ - Spearman correlation coefficient

regression analyses (Tables 3 and 4). Higher plasma resistin concentrations, lower resistin mRNA, and increased CAP-1 mRNA levels were singled out as possible predictors of increased risk for CRC development in the univariate analysis, although a wide confidence interval (most likely due to smaller sample size) was reached in the case of CAP-1 mRNA. After adjustment for confounding variables—age, gender, BMI, HDL-C, LDL-C in multivariate analysis (Table 4)—CAP-1 mRNA lost its predictive potential, while plasma resistin remained statistically significant independent predictor of increased risk for CRC development. However, the assessed odds ratio for resistin (OR = 1.055; 95% CI 1.009–1.103) implies limited contribution of this single parameter to overall CRC risk, whereas the entire model showed satisfying statistical performances. Omnibus Tests of Model Coefficients (goodness of fit test) showed high statistical significance ($p < 0.001$) with χ^2 value of 82.314 for 7 degrees of freedom (*df*). Hosmer-Lemeshow goodness of fit test, for constructed model, was satisfying ($\chi^2 = 7.252$; $df = 8$; $p = 0.510$), while value of Nagelkerke R Square was 0.596. The obtained accuracy in classification was 82.7%. Normalized resistin mRNA was not included in a model since it did not satisfy Hosmer-Lemeshow goodness of fit requirement in univariate logistic regression ($p < 0.001$).

Next, we estimated clinical accuracy of investigated parameters and clusters of parameters (Table 5). AUC > 0.7 was obtained for all investigated markers except for CAP-1 mRNA, while AUC > 0.8 was obtained for plasma resistin/resistin mRNA ratio and combination of all independent predictors of CRC identified in previous logistic regression analysis (age, BMI, HDL-C, LDL-C, and plasma resistin concentrations).

Finally, we analyzed the association among *RETN* polymorphism rs1862513 and other examined resistin-linked parameters. Hardy-Weinberg's equilibrium existed in both our cohorts [CRC group: $\chi^2 = 1.196$ $p = 0.274$ for subsequent genotype distribution (expressed as relative frequencies) CC = 0.55; CG = 0.35; GG = 0.1], [control group: $\chi^2 = 0.525$ $p =$

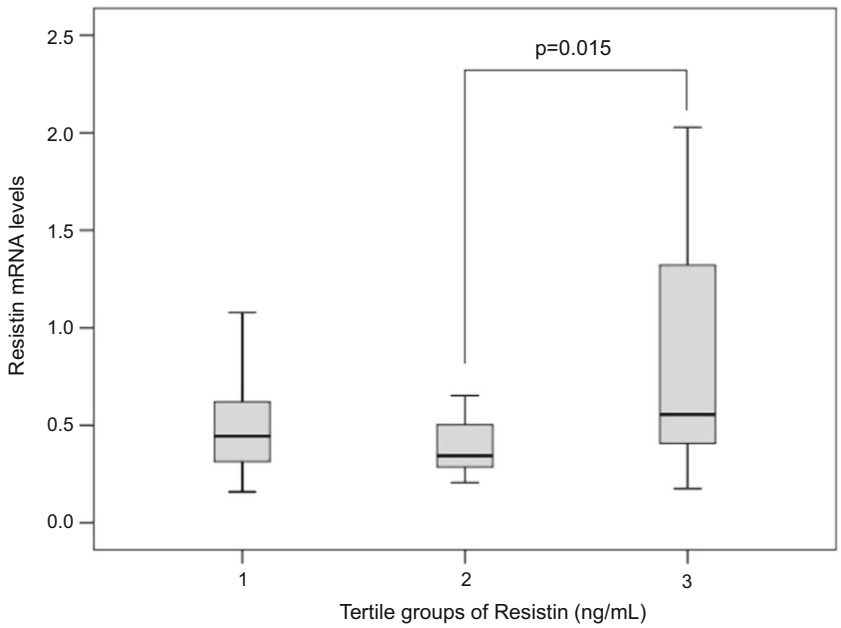

Fig. 1 Changes in PBMC resistin mRNA levels across tertile of plasma resistin concentration in patients with colorectal cancer

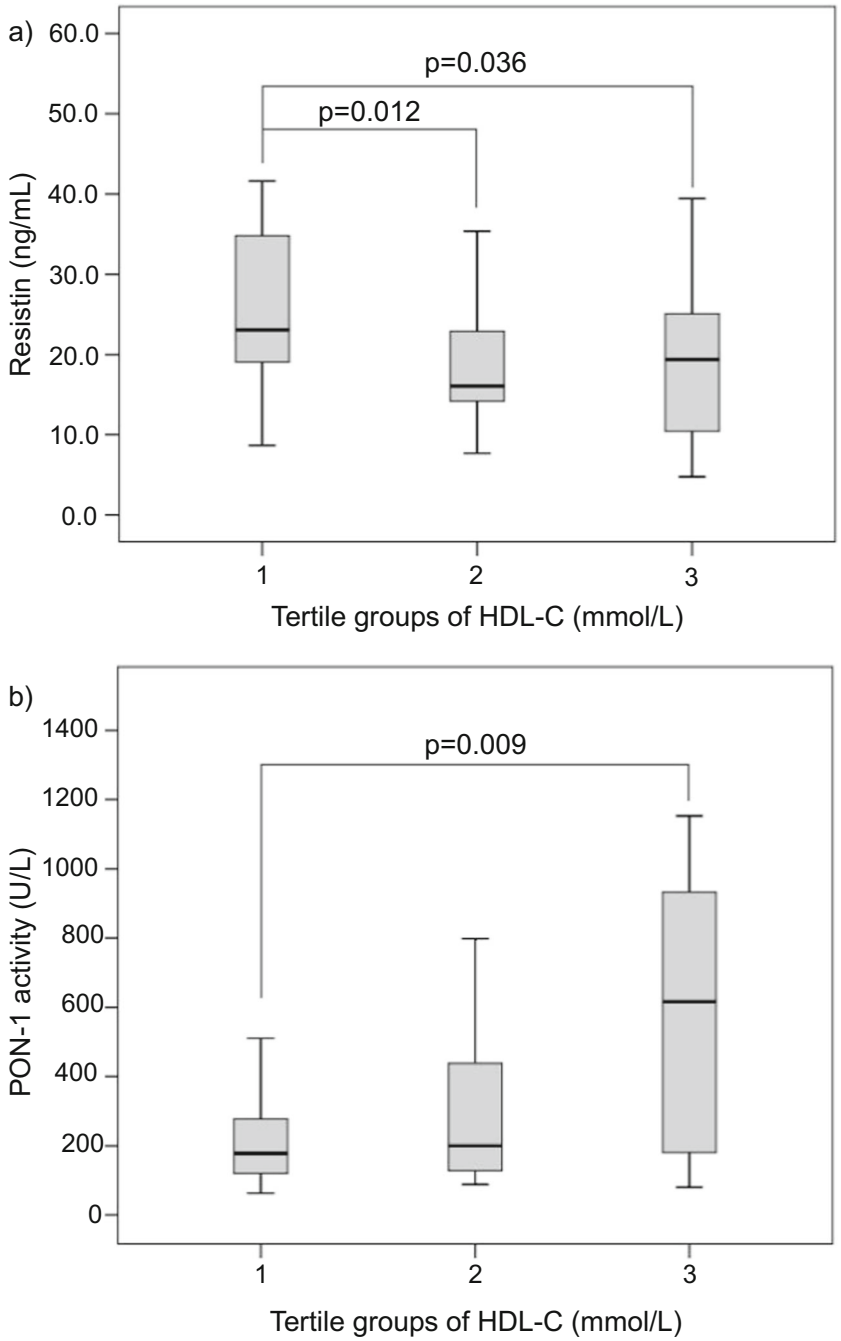

Fig. 2 Changes in plasma resistin concentrations and PON1 activities across tertile of HDL-C concentration in patients with colorectal cancer. **a**) Changes in plasma resistin concentrations across tertile of HDL-C concentration. **b**) Changes in PON-1 activities across tertile of HDL-C concentration

0.469 for subsequent genotype distribution (expressed as relative frequencies) CC = 0.57; CG = 0.35; GG = 0.08]. CRC and control groups did not differ regarding genotype distribution ($\chi^2 = 0.210$; $p = 0.900$). Kruskal-Wallis test demonstrated that there were no differences in resistin plasma concentration, resistin mRNA, and CAP-1 mRNA levels among subjects with CC, CG, and GG genotypes, neither in CRC patients (resistin: $p = 0.361$; resistin mRNA: $p = 0.509$; CAP-1 mRNA: $p = 0.147$), nor in the control group (resistin: $p = 0.439$; resistin mRNA: $p = 0.074$; CAP-1 mRNA: $p = 0.860$).

Discussion

In this study, the relationship among plasma resistin concentration, resistin mRNA, and CAP-1 mRNA levels were investigated for the first time in CRC patients. Our results showed higher plasma resistin concentration, followed by lower resistin mRNA levels in CRC patients. Additionally, higher CAP-1 mRNA was demonstrated in CRC group.

Recently, a study performed on monocytes suggested that CAP-1 is a receptor for human resistin [17], but a limited number of studies have investigated resistin-CAP-1

Table 3 Univariate binary logistic regression analysis for the associations of plasma resistin concentration, normalized resistin mRNA and CAP1 mRNA levels with the risk for CRC onset

Parameter	OR	95% CI	*p*
Resistin (ng/mL)	1.071	1.031–1.112	< 0.001
Normalized resistin mRNA	0.325	0.160–0.661	< 0.010
Normalized CAP-1 mRNA	4.476	1.124–17.824	< 0.050

Variables are entered as continuous

interactions in malignant diseases. In contrast, the effects mediated by the association of resistin with TLR4 were extensively reviewed. Wang et al. have demonstrated that resistin-TLR4 interaction in carcinoma cell lines has tumor-promoting effects during the development of breast cancer [24]. It was also suggested that in gastric carcinoma cells, resistin stimulates angiogenesis through TLR4-mediated effects [25]. Nevertheless, it has been shown that immunohistochemical staining for CAP-1 protein is positive in over 50% of CRC tissue samples [26], thus implicating that CAP-1-resistin interaction might play important role in this disease development. It has been already demonstrated that CAP-1 tissue mRNA overexpression is associated with lower surviving rate in breast and ovarian cancers [27]. As an actin binding protein [28], CAP-1 was considered as a metastasis promoting factor, as well as cancer invasiveness mediator [26, 27]. In our current study, CAP-1 mRNA levels were evaluated and found to be higher in CRC patients (Table 1). Concomitantly higher plasma resistin concentrations (Table 1) might indicate significant resistin-CAP-1 interaction in CRC, which should be further explored. Based on our preliminary findings in CRC patients, we could hypothesize a presence of a characteristic pattern of resistin-CAP-1 interaction, which is different from well-known resistin-TRL4 cooperation and might be of potential interest as either biomarker or therapeutic target. It has been previously demonstrated that association of resistin with CAP-1 in monocytes leads to increased production of pro-

Table 4 Multivariate binary regression analysis for independent associations of plasma resistin concentration with risk for development of CRC

Parameter	OR	95% CI	*p*
Age (years)	1.132	1.071–1.196	< 0.001
Gender (f/m)	1.366	0.477–3.907	0.561
BMI (kg/m^2)	0.800	0.688–0.931	< 0.010
HDL-C (mmol/L)	0.263	0.092–0.748	< 0.050
LDL-C (mmol/L)	0.449	0.282–0.715	< 0.001
Resistin (ng/mL)	1.055	1.009–1.103	< 0.050
Normalized CAP-1 mRNA	5.409	0.645–45.376	0.120

Variables are entered as continuous, except for gender. Enter model

Table 5 Areas of ROC (AUC) for estimation of clinical accuracy of examined parameters

Parameter	AUC	CI	*p*
Resistin (ng/mL)	0.741	0.663–0.820	< 0.001
Normalized CAP-1 mRNA	0.603	0.515–0.690	< 0.050
Plasma resistin/resistin mRNA	0.821	0.754–0.889	< 0.001
Model 1	0.748	0.668–0.828	< 0.001
Model 2	0.757	0.671–0.843	< 0.001
Model 3	0.898	0.845–0.950	< 0.001

Model 1: normalized CAP-1 mRNA + resistin (ng/mL)

Model 2: resistin (ng/mL) + PON1 activity (U/L) + HDL-C (mmol/L)

Model 3: included all independent predictors of CRC as detected by multivariate logistic regression analysis (age (years) + BMI (kg/m^2) + HDL-C (mmol/L) + LDL-C (mmol/L) + resistin (ng/mL))

inflammatory cytokines [17]. Since these cytokines enhance PBMC resistin mRNA expression [29], a positive feedback loop can be made, thereby further promoting pro-inflammatory environment necessary for the onset of CRC.

Although pro-inflammatory role of resistin is especially emphasized in numerous studies, its negative correlation with HDL-C was also observed in different pathological conditions, including CRC [30]. This correlation was singled out as a significant one in our patients as well (Table 2; Fig. 2a). The mechanism through which HDL can inhibit the production of resistin has already been proposed. Namely, it has been shown in 3T3L1 murine adipocytes that HDL particles interact with oxidized LDL causing lower free cholesterol accumulation and reduced endoplasmic reticulum stress, which consequently leads to decreased adipocytokine secretion [31]. Although significant discrepancy among human and mouse resistin prevents deriving definitive conclusions, these findings suggest that elevation of circulating resistin levels in humans might arise as a consequence of decreased HDL-C and impaired HDL particle functionality. In our previous study, we demonstrated that HDL particle distribution in patients with CRC is characterized by lower diameter and increased proportion of smaller HDL3b particles, implying compromised HDL functionality [13]. Accordingly, in the current study, we found a change in PON-1 activity across tertiles of HDL-C (Fig. 2b). Since PON-1 is an HDL-associated enzyme, responsible for degradation of oxidized lipids within LDL, it can be hypothesized that dysfunctional HDL particles in CRC are unable to prevent an increase in resistin secretion. On the other hand, resistin promotes formation of pro-inflammatory environment, which can further diminish HDL functionality [32], thus contributing to aggravation of the initial pathological process. Recently, Lu and Zhan pointed out to the importance of multi-parameter model in understanding of cancer complexity and detecting of reliable biomarkers [14]. The observed association among plasma

resistin, HDL-C, and PON-1 activity in CRC confirms such point of view and underline its importance for better understanding, and consequently, efficient prediction and prevention of CRC development.

Although macrophages are the main source of plasma resistin in humans, PBMCs, namely monocytes and lymphocytes, also produce resistin and thereby contribute to overall plasma resistin concentration [33]. However, determination of PBMC resistin mRNA levels in our study yielded unexpected results. In contrast to higher plasma resistin concentration, we have found lower PBMC resistin mRNA levels in CRC patients (Table 1). A possible reason for such discrepancy could be found in diverse resistin productions by PBMCs and malignant tissue. It has been suggested that increased resistin secretion in CRC could be attributed to tumor-infiltrating macrophages, rather than to malignant cells themselves [34]. Additionally, a recent study in women with breast cancer demonstrated that serum resistin concentration was not correlated with its gene expression in cancerous tissue [35]. In contrast to a longstanding opinion according to which macrophages originates exclusively from blood monocytes, it has been demonstrated that precursors of tissue-resident macrophages are progenitor cells from yolk sac and fetal liver [36]. On the other side, monocyte-derived macrophages become abundant in tissues during inflammatory response [36]. Having in mind that chronic inflammation is associated with CRC [37], it can be assumed that the observed higher plasma resistin levels in our patients arise as a consequence of its increased production by highly abundant tumor-associated macrophages. Yet, we found a borderline significant positive correlation of resistin mRNA with plasma resistin concentration in CRC patients (Table 2). In addition, we observed an increase in resistin mRNA across tertiles of plasma resistin (Fig. 1). Altogether, we can presume that PBMCs are contributors to circulating resistin level, but not the only ones and likely not the most significant ones in CRC. The obtained results led us to the hypothesis that the discrepancy between plasma resistin and PBMC mRNA resistin levels might be used as a potential indicator of CRC development. We therefore calculate and analyzed plasma resistin/resistin mRNA ratio. As a confirmation of our hypothesis, we found significantly higher values of plasma resistin/resistin mRNA ratio in CRC patients (Table 1). Furthermore, this ratio exhibited high discriminative abilities for CRC (Table 5). Such findings might have important consequences for potential use of resistin protein and mRNA levels in diagnosis and prognosis of CRC. Namely, increased plasma resistin and plasma resistin/resistin mRNA ratio might be considered as easily detectable candidate markers of CRC development and progression. Thus, methods of molecular biology and genetic tests can complement and improve traditional biochemical determination of protein levels, as already postulated [38]. Additionally, this ratio can be easily determined in samples obtained by a simple venepuncture, which could present a significant advantage for potential clinical use. Nevertheless, our preliminary findings should be further tested and evaluated by basic and clinical studies.

In spite of still unresolved questions regarding the exact role of resistin in colorectal carcinogenesis, higher resistin levels in CRC are reported in large number of studies [18]. An association among tumor stage and serum resistin concentrations has been shown [30] and possible use of resistin as a marker for CRC was emphasized [39]. In light of these evidences, we examined the predictive potential of plasma resistin, PBMC resistin mRNA and CAP-1 mRNA levels for CRC development. All three parameters individually had a significant predictive potential (Table 3), while in multivariate binary logistic regression, which included age, gender, BMI, LDL-C, HDL-C, and normalized CAP1 mRNA as confounders, resistin remained a significant predictor of CRC risk (Table 4). Although the obtained odds ratio (OR = 1.055; 95% CI 1.009–1.103) suggests minor involvement of plasma resistin in elevation of CRC risk, the obtained statistical significance still indicates independent contribution of resistin to the development of this disease. In addition, our preliminary findings demonstrated that clinical accuracy of resistin was comparable with currently used tumor markers for CRC, such as serum CA 19-9 and CEA [40]. Moreover, clustering of resistin with resistin mRNA and several easily obtainable anthropometric and laboratory data (age, BMI, LDL-C and HDL-C) yielded sufficiently high clinical accuracy (Table 5), emphasizing the importance of a multi-marker approach in prediction and diagnosis of CRC. Taken altogether, resistin protein and mRNA levels might be proposed as candidate biomarkers for CRC, which should be further evaluated.

Previous studies linked *RETN* polymorphism rs1862513 (− 420 C/G) with higher concentration of resistin and consequently with aggravated pro-inflammatory status in patients with CRC and breast cancer, emphasizing GG allelic variant of gene as a dominant for these effects [22]. Osawa et al. showed the association among *RETN* rs1862513 polymorphism in promoter region and enhanced resistin mRNA expression due to the altered binding of Sp1/3 transcriptional factors [41]. However, the same authors suggested the presence of other significant SNPs, proposing that multiple effects of different alleles have to be taken into account [42]. Additionally they demonstrated that methylation at SNP-420 was associated with lower resistin's concentration in CC and CG genotypes [43]. In our current study, we did not find any association of altered resistin and CAP-1 gene expression levels or plasma resistin concentration with *RETN* rs1862513 polymorphism. In addition, we found no differences regarding allelic distribution in CRC patients and healthy individuals. Taken altogether, we can assume that lack of association among this specific SNP and resistin gene expression and protein concentration in our CRC patients might

be a consequence of other genetic or epigenetic effects that go beyond a single genetic change, emphasizing the importance of more comprehensive approach to genetic analyses.

Several limitations should be mentioned. First, the observed discrepancy among plasma resistin and PBMCs resistin mRNA levels cannot be fully explained without an insight into resistin concentration and gene expression in cancerous tissue. In the current study, we used blood samples, so we could not perform such analyses. Also, comparison of our current results with findings of previous studies is partially limited by the fact that majority of other studies utilized tissue samples or cell cultures. However, a plausible use of resistin as a prognostic marker would include less invasive techniques, such is venepuncture, and thus investigations in blood samples are rationale and needed. Next, the observational study design does not allow drawing a conclusion regarding causative relationship among resistin level and CRC development. In addition, relatively small sample size limited deriving firm conclusions regarding the observed associations. Longitudinal studies with larger sample size are needed to verify our preliminary findings.

Conclusions and expert recommendations

Our study demonstrated higher plasma resistin and PBMCs CAP-1 mRNA levels in patients with CRC when compared to healthy individuals, implying their possible interaction during the onset of this malignancy. Lack of concomitant increase in resistin mRNA levels suggests that overproduction of resistin in CRC is related to other sources, most likely tumor-associated macrophages, than to circulating PBMCs. In addition, plasma resistin concentration and plasma resistin/PBMC resistin mRNA ratio were revealed as potentially useful diagnostic and prognostic tools. Finally, the observed associations among resistin and HDL-C raise the possibility that a delicate interplay among these two markers of dyslipidemia and inflammation participates in pathogenesis of CRC, which should be further explored and evaluated by future studies.

In our research, we used plasma and PBMCs collected by a simple venepuncture, which is minimally invasive, rapid, and inexpensive procedure for acquiring of appropriate and representative samples. Our results suggest that such easily obtainable samples could provide clusters of integrated biomarkers related to CRC, with significant predictive abilities and satisfying clinical accuracy. Therefore, minimally onerous and relatively comfortable sampling procedure could ensure multimarker strategy which is a hallmark of preventive, predictive, and personalized medicine practice. Additionally, our findings implicate that evaluation of protein concentration should be complemented with molecular detection, in order to obtain an insight into possible deregulation regarding protein coding, posttranscriptional and posttranslational modifications and interactions with receptors.

In summary, resistin protein and PBMC mRNA levels might be considered as potential candidate biomarkers for CRC. Further evaluation of these parameters is necessary to assure the most reliable conclusion regarding the implementation of these analyses in routine clinical laboratories. In addition to all requirements of good laboratory practice, a cost-efficacy analysis is needed for precise estimation of benefits that could be achieved by implementation of these markers.

Authors' contribution MM (corresponding author) performed laboratory measurements, statistical analysis, and wrote the first draft of the manuscript. AN, MS, MM, and AS contributed to experimental design and participated in laboratory analyses. DZ, BT, and ZS were involved in protocol development, patient recruitment, and data acquisition. JV and VSK provided intellectual guidance and critically reviewed the manuscript. AZ conceived and designed the study and critically reviewed the manuscript. All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Funding information This work was supported by a grant from the Ministry of Education, Science and Technological Development, Republic of Serbia (Project No. 175035).

Compliance with ethical standards

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

Ethical approval The entire study protocol was designed according to the Helsinki Declaration and approved by the local ethical committee (The Ethics committee of the Military Medical Academy; Ethics Committee reference no. 3000-1).

References

1. Nyström M, Mutanen M. Diet and epigenetics in colon cancer. World J Gastroenterol. 2009;15:257–63.
2. Ma Y, Yang Y, Wang F, Zhang P, Shi C, Zou Y, et al. Obesity and risk of colorectal cancer: a systematic review of prospective studies. PLoS One. 2013;8:e53916.
3. Grech G, Zhan X, Yoo BC, Bubnov R, Hagan S, Danesi R, et al. EPMA position paper in cancer: current overview and future perspectives. EPMA J. 2015;6:9.
4. Schwartz DR, Lazar MA. Human resistin: found in translation from mouse to man. Trends Endocrinol Metab. 2011;22:259–65.
5. İclal G, Mehmet K, Yılmaz K, Müslüm A. Increased resistin serum concentrations in patients with type 1 diabetes mellitus. J Clin Res Pediatr Endocrinol. 2013;5:189–93.
6. Asgary S, SamsamShariat S, Ghorbani A, Keshvari M, Sahebkar A, Sarrafzadegan N. Relationship between serum resistin concentrations with metabolic syndrome and its components in an Iranian population. Diabetes Metab Syndr Clin Res Rev. 2015;9:266–70.
7. Konrad A, Lehrke M, Schachinger V, Seibold F, Stark R, Ochsenkühn T, et al. Resistin is an inflammatory marker of inflammatory bowel disease in humans. Eur J Gastroenterol Hepatol. 2007;19:1070–4.
8. Riondino S, Roselli M, Palmirotta R, Della-Morte D, Ferroni P, Guadagni F. Obesity and colorectal cancer: role of adipokines in

tumor initiation and progression. World J Gastroenterol. 2014;20:5177–90.
9. Feng Z, Zhang H. Resistin and cancer risk: a mini-review. Endocrinol Metabol Syndrome. 2011;S4:003.
10. Dalamaga M. Resistin as a biomarker linking obesity and inflammation to cancer: potential clinical perspectives. Biomark Med. 2014;8:107–18.
11. Yao X, Tian Z. Dyslipidemia and colorectal cancer risk: a meta-analysis of prospective studies. Cancer Causes Control. 2015;26:257–68.
12. Mihajlovic M, Gojkovic T, Vladimirov S, Miljkovic M, Stefanovic A, Vekic J, et al. Changes in lecithin: cholesterol acyltransferase, cholesteryl ester transfer protein and paraoxonase-1 activities in patients with colorectal cancer. Clin Biochem. 2019;63:32–8.
13. Stevanovic M, Vekic J, Bogavac-Stanojevic N, Janac J, Stjepanovic Z, Zeljkovic D, et al. Significance of LDL and HDL subclasses characterization in the assessment of risk for colorectal cancer development. Biochem Med. 2018;28:503–13.
14. Lu M, Zhan X. The crucial role of multiomic approach in cancer research and clinically relevant outcomes. EPMA J. 2018;9:77–102.
15. Golubnitschaja O, Costigliola V. General report & recommendations in predictive, preventive and personalised medicine 2012: white paper of the European Association for Predictive, Preventive and Personalised Medicine. EPMA J. 2012;3:14.
16. Tarkowski A, Bjersing J, Shestakov A, Bokarewa M. Resistin competes with lipopolysaccharide for binding to toll-like receptor 4. J Cell Mol Med. 2010;14:1419–31.
17. Lee S, Lee HC, Kwon YW, Lee SE, Cho Y, Kim J, et al. Adenylyl cyclase-associated protein 1 is a receptor for human resistin and mediates inflammatory actions of human monocytes. Cell Metab. 2014;19:484–97.
18. Yang G, Fan W, Luo B, Xu Z, Wang P, Tang S, et al. Circulating resistin levels and risk of colorectal cancer: a meta-analysis. Biomed Res Int. 2016;2016:1–11.
19. Munjas J, Sopić M, Spasojević-Kalimanovska V, Kalimanovska-Oštrić D, Anđelković K, Jelić-Ivanović Z. Association of adenylate cyclase-associated protein 1 with coronary artery disease. Eur J Clin Investig. 2017;47:659–66.
20. Cheng T, Zhan X. Pattern recognition for predictive, preventive, and personalized medicine in cancer. EPMA J. 2017;8:51–60.
21. Golubnitschaja O, Baban B, Boniolo G, Wang W, Bubnov R, Kapalla M, et al. Medicine in the early twenty-first century: paradigm and anticipation - EPMA position paper. EPMA J. 2016;7:23.
22. Hashemi M, Bahari G, Tabasi F, Moazeni-Roodi A, Ghavami S. Association between rs1862513 and rs3745367 genetic polymorphisms of resistin and risk of cancer: a meta-analysis. Asian Pac J Cancer Prev. 2018;19:2709–16.
23. Vujovic A, Spasojevic-Kalimanovska V, Bogavac-Stanojevic N, Spasic S, Kotur-Stevuljevic J, Jelic-Ivanovic Z. Comparison of two RNA isolation methods for determination of SOD1 and SOD2 gene expression in human blood and mononuclear cells. Indian J Biotechnol. 2013;12:468–74.
24. Wang CH, Wang PJ, Hsieh YC, Lo S, Lee YC, Chen YC, et al. Resistin facilitates breast cancer progression via TLR4-mediated induction of mesenchymal phenotypes and stemness properties. Oncogene. 2018;37:589–600.
25. Hsieh YY, Shen CH, Huang WS, Chin CC, Kuo YH, Hsieh MC, et al. Resistin-induced stromal cell-derived factor-1 expression through toll-like receptor 4 and activation of p38 MAPK/ NFκB signaling pathway in gastric cancer cells. J Biomed Sci. 2014;21:59.
26. Zhang H, Zhou G. CAP1 (cyclase-associated protein 1) exerts distinct functions in the proliferation and metastatic potential of breast Cancer cells mediated by ERK. Sci Rep. 2016;6:25933.
27. Xie S, Shen C, Zhou S, Xiong M, Tan M, Song X, et al. Role of adenylate cyclase-associated protein 1 in cancers. Int J Clin Exp Med. 2018;11:3086–99.
28. Ono S. The role of cyclase-associated protein in regulating actin filament dynamics–more than a monomer-sequestration factor. J Cell Sci. 2013;126:3249–58.
29. Kaser S, Kaser A, Sandhofer A, Ebenbichler CF, Tilg H, Patsch JR. Resistin messenger-RNA expression is increased by proinflammatory cytokines in vitro. Biochem Biophys Res Commun. 2003;309:286–90.
30. Danese E, Montagnana M, Minicozzi AM, Bonafini S, Ruzzenente O, Gelati M, et al. The role of resistin in colorectal cancer. Clin Chim Acta. 2012;413:760–4.
31. Song G, Wu X, Zhang P, Yu Y, Yang M, Jiao P, et al. High-density lipoprotein inhibits ox-LDL-induced adipokine secretion by upregulating SR-BI expression and suppressing ER stress pathway. Sci Rep. 2016;6:30889.
32. Rosso LG, Lhomme M, Merono T, Sorroche P, Catoggio L, Soriano E, et al. Altered lipidome and antioxidative activity of small, dense HDL in normolipidemic rheumatoid arthritis: relevance of inflammation. Atherosclerosis. 2014;237:652–60.
33. Kunnari AM, Savolainen ER, Ukkola OH, Kesäniemi YA, Jokela MA. The expression of human resistin in different leucocyte lineages is modulated by LPS and TNFalpha. Regul Pept. 2009;157:57–63.
34. Ghaemmaghami S, Mohaddes S, Hedayati M, Mohammadi M, Dehbashi G. Resistin and visfatin expression in HCT-116 colorectal cancer cell line. Int J Mol Cell Med. 2013;2:143–50.
35. Munoz-Palomeque A, Guerrero-Ramirez MA, Rubio-Chavez LA, Rosales-Gomez RC, Lopez-Cardona MG, Barajas-Avila VH, et al. Association of RETN and CAP1 SNPs, expression and serum resistin levels with breast cancer in Mexican women. Genet Test Mol Biomarkers. 2018;22:209–17.
36. Gordon S, Plüddemann A. Tissue macrophages: heterogeneity and functions. BMC Biol. 2017;15:53.
37. Wang K, Karin M. Tumor-elicited inflammation and colorectal cancer. Adv Cancer Res. 2015;128:173–96.
38. Janssens JP, Schuster K, Voss A. Preventive, predictive, and personalized medicine for effective and affordable cancer care. EPMA J. 2018;9:113–23.
39. Nakajima TE, Yamada Y, Hamano T, Furuta K, Matsuda T, Fujita S, et al. Adipocytokines as new promising markers of colorectal tumors: adiponectin for colorectal adenoma, and resistin and visfatin for colorectal cancer. Cancer Sci. 2010;101:1286–91.
40. Ning S, Wei W, Li J, Hou B, Zhong J, Xie Y, et al. Clinical significance and diagnostic capacity of serum TK1, CEA, CA 19-9 and CA 72-4 levels in gastric and colorectal cancer patients. J Cancer. 2018;9:494–501.
41. Osawa H, Yamada K, Onuma H, Murakami A, Ochi M, Kawata H, et al. The G/G genotype of a resistin single-nucleotide polymorphism at −420 increases type 2 diabetes mellitus susceptibility by inducing promoter activity through specific binding of Sp1/3. Am J Hum Genet. 2004;75:678–86.
42. Osawa H, Tabara Y, Ohashi J, Kawamura R, Onuma H, Makino H. Is rs34861192 or rs1862513 a more promising variant for determining plasma resistin in an aged Japanese population? Diabetologia. 2010;53:795–7.
43. Onuma H, Tabara Y, Kawamura R, Ohashi J, Nishida W, Takata Y, et al. Dual effects of a RETN single nucleotide polymorphism (SNP) at −420 on plasma resistin: genotype and DNA methylation. J Clin Endocrinol Metab. 2017;102:884–92.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.