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Original Article

Plasma soluble receptor for advanced glycation end-products and risk of colorectal adenoma

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Abstract: Receptor for advanced glycation end products (RAGE) plays an important role in promoting chronic inflammation with activation of NF-κB. Soluble form of RAGE (sRAGE) represents a naturally occurring competitive inhibitor of RAGE-mediated events. In a colonoscopy-based case-control study, we examined the associations of plasma levels of sRAGE, sTNF-αRI, sTNF-αRII, sIL-6R, EGF, IFNα2, G-CSF, MCP1, TNFβ, and VEGF with risk of colorectal adenoma. We prospectively identified 158 cases with colorectal adenoma and 203 polyp-free controls who were frequency-matched according to age, sex, race, and time of blood draw. Exposure information was collected using a questionnaire and fasting plasma samples were obtained before the colonoscopy. We used Luminex bead-based multiplex assays to determine level of biomarkers. Multivariate logistic regression model was used to estimate odds ratio (OR) and its 95% confidence interval (CI). Cases had insignificant lower levels of sRAGE, and higher levels of EGF and VEGF than controls. When the highest compared with the lowest category, the OR (95% CI) of colorectal adenoma was 0.55 (0.31-0.96) (*P* trend = 0.03) for sRAGE and 1.75 (1.05-2.93) (*P* trend =0.04) for VEGF, adjusting for age, smoking status, hypertension and type 2 diabetes. The inverse association between sRAGE and colorectal adenoma was seen only among those without hypertension (*P* interaction = 0.02). An inverse association between sRAGE and colorectal adenoma was in line with an inverse association between sRAGE and colorectal cancer previously reported. This study supported the involvement of RAGE-NF-kB related inflammatory mechanism in the formation of colorectal adenoma.

Keywords: Case-control, colorectal adenoma, risk, inflammation, sRAGE, VEGF, NF-kB

Introduction

Chronic inflammation is one of the hallmarks of colorectal cancer. However, although most colorectal cancer arises from sporadic colorectal adenomas, our understanding on the role of inflammation in the formation of colorectal adenomas is limited. Obesity and type 2 diabetes, conditions that are characterized by chronic systemic inflammation, are risk factors for colorectal cancer and possibly for colorectal adenoma [1]. Recent prospective cohort studies and case-control studies showed that circulating levels of inflammatory biomarkers, including tumor necrosis factor (TNF)-α, C-reactive protein (CRP) and interleukin 6 (IL-6) [2-5], are predicative of the occurrence or recurrence of colorectal adenomas.

Receptor for advanced glycation end-products (RAGE) is a member of immunoglobulin superfamily that serves as a transmembrance multiligand receptor. Its main ligands include advanced glycation end-products (AGEs), S100/calgranulins (such as S100P and S100A4), and high-mobility group box 1 protein (HMGB1) [6]. The engagement of full length RAGE with its ligands triggers rapid generation of intracellular reactive oxygen species and activates an array of cell signaling pathways that lead to the activation of the transcription factor NF-κB [7]. RAGE has been shown to plays a role in intestinal inflammation and tumorigenesis in experimental studies [8, 9]. RAGE has also been associated with invasion, metastasis, and poor prognosis of colorectal cancer in clinical studies [10].

Soluble receptor for advanced glycation end-products (sRAGE) can also bind the ligands of RAGE. However, sRAGE lacks the intracellular signaling domain, and therefore its engagement with ligands does not trigger signal transduction. sRAGE therefore functions as a decoy receptor for RAGE ligands that neutralizes the effects mediated by full-length RAGE. We previously found in a prospective cohort study of Finnish male smokers that higher baseline circulating sRAGE was associated with lower risk of developing colorectal cancer [11]. This observation indicated that RAGE-related mechanisms contribute to an inflammatory microenvironment and consequently colorectal carcinogenesis. However, the role of sRAGE in colorectal adenomas is unknown.

In this present case-control study, we examined the association between plasma levels of sRAGE as well as soluble receptors for inflammatory cytokines including soluble tumor necrosis factor receptor I (sTNFRI) and II (sTNFRII), soluble interleukin-6 receptor (sIL-6R), and cytokines that are downstream of RAGE, including epidermal growth factor (EGF), granulocyte colony-stimulating factor (G-CSF), interferon α2 (IFNα2), monocyte chemotactic protein-1 (MCP1), tumor necrosis factors β (TNF-β), and vascular endothelial growth factor (VEGF) and the risk of colorectal adenomas.

Materials and methods

Study design and study population

We conducted a frequency-matched case-control study among individuals age 50 to 75 years presenting for screening or surveillance colonoscopy at the Michael E. DeBakey VA Medical Center (MEDVAMC) between September 2008 and April 2011. Individuals were eligible to participate in the study if they received a complete colonoscopy and provided informed consent. The exclusion criteria before the colonoscopy were: 1) suspected cancer or severe feeding difficulties or unintentional weight loss more than 10 lbs during the past 3 months; 2) previous gastroesophageal surgery, iron-deficiency anemia, or malignancy of the esophagus or stomach; and 3) contraindication to biopsy such as bleeding disorders or use of anticoagulants or antiplatelets. Additional exclusion criteria after the colonoscopy included individuals who 1) did not provide whole blood or complete the study interview; 2) had a history or finding of colorectal cancer, familial adenomatous polyposis, hereditary nonpolyposis colon cancer, or inflammatory bowel diseases; 3) had hyperplastic polyps according to the pathological examination; and 4) had serum creatinine levels greater than 1.5 mg/dL. The reason for this exclusion is that previous studies found that sRAGE levels are significantly elevated in patients with end-stage renal disease [12].

Case subjects were defined as individuals who had at least one colorectal adenomatous polyp, and control subjects were defined as individuals with no colorectal polyps of any type or prior history of polyps. Among 500 study subjects enrolled from the colonoscopy clinic, we excluded 10 because of an insufficient blood sample, 6 who did not complete study questionnaires, 65 with hyperplastic polyps only, 26 subjects who had non-adenoma polyps, 5 with adenocarcinoma, 4 with diminutive adenomatous change, and 18 (6 cases and 12 controls) with serum creatinine levels greater than 1.5 mg/dL. We further excluded 5 polyp-free controls who could not be matched to cases. The biomarkers of interest were measured among 361 study subjects, including 158 cases with adenoma and 203 polyp-free controls. After the Luminex bead-based multiplex assays, 4 measurement outliers (± 2 standard deviation of mean) for sRAGE and 8 for EGF were not included in the respective analysis. Cases and controls were frequency matched according to age (± 2 years), sex, race, and time of blood draw (± 4 months).

This study was approved by the Institutional Review Boards at both Baylor College of Medicine and the MEDVAMC.

Data and biospecimens

Before the colonoscopy procedure, a trained research assistant administered a computer-assisted study questionnaire to collect information on demographic features, lifestyles, family history of cancer, medications, medical history (such as diabetes and hypertension) and physical activity. Never smokers were defined as individual who never smoked more than 100 cigarettes in whole life. Formers smokers were defined as individuals who were ever smokers but did not smoke during the past year. Physical activity was classified to low, moderate, and

high according to metabolic equivalent of task (MET) for recreational activity. Having diabetes was defined as self-reported treatment of diabetes with insulin injection or tablets and/or diet. Self-reported high blood pressure (hypertension) was recorded. The research assistant also obtained anthropometric measurements (height, weight, waist and hip circumference) before the colonoscopy. We obtained information on serum levels of creatinine, histology type (tubular, tubulovillous, villous, or serrated), location, and size of adenoma from the colonoscopy and pathological reports. The pathological feature of the most advanced or largest adenoma was documented if the study subjects had multiple adenomas.

Whole blood specimen was collected before the colonoscopy after an overnight fast. The specimens were centrifuged and aliquoted within 2 hours of blood draw. The isolated plasma samples were stored at -80°C until use. None of the samples went through more than two freeze-thaw cycles before the lab assay.

Luminex bead- based multiplex assay

We measured 10 circulating protein biomarkers in duplicate using two multiplex panels from Millipore (EMD Millipore Corporation, Billerica, MA, USA). The first panel measured plasma levels of 4 soluble receptors, including sRAGE, sTNF-αRI, sTNF-αRII, and sIL-6R; and the second panel measured 6 human cytokines/chemokine, including EGF, IFNα2, G-CSF, TNFβ, MCP1, and VEGF. The samples from the cases and the frequency-matched controls were strictly placed on the same plate. The samples were also placed proportionally on the multiplex microplate. We included on each plate a 3% quality control (QC) plasma sample pooled from patients who did not provide the questionnaire data. For the assay of soluble receptor, the plasma sample was 1:2 diluted. For the assay of cytokines, the plasma samples were not diluted. The assay was performed in the Dan L. Duncan Cancer Center Proteomics core lab using Bio-Plex 200 system (Bio-Rad, Hercules, CA) and the data were analyzed by Bio-Plex manager 6.0 software with 5PL curve fitting, as per the manufacturer's instructions. On the day of the experiment, the plates with samples were thawed in a water bath under room temperature and spun at 900 xg for 3 minutes. The plates were then spun down at 1,000 xg for 5 minutes and then aliquoted onto the 96-well filter plate for assay incubation. Laboratory personnel who handled the samples were blinded to the case, control and QC status of the samples. The assays were completed by one personnel within a 6-week period using the same lot of reagents and the overnight protocol was used to improve the sensitivity of the assay. The lowest detection limit (pg/ml) (minimum detectable concentration) was 4.7 for sRAGE, 9.6 for sTNF-αRI, 16.9 for sTNF-αRII, 3.6 for sIL-6R, 2.7 for EGF, 24.5 for IFNα2, 0.5 for G-CSF, 1.9 for TNFβ, 0.9 for MCP1, and 5.8 for VEGF. For sRAGE, we also used human sRAGE Quantikine ELISA kit (R&D system Inc, Minneapolis, MN) to test 11 samples that were tested in the multiplex assay. This sRAGE Quantikine ELISA kit was used in our previous study on sRAGE and colorectal cancer [11].

Statistical analysis

Medians and intertertile ranges for each biomarker were calculated. Precision of each plate was assessed by using intra- and inter-plate coefficient of variation (CV) using blinded QC samples. Spearman's rank test was used to evaluate correlations between plasma levels of biomarkers among controls.

Demographic and clinical characteristics were compared between cases and controls, and χ^2 test was used to assess differences in proportions. Levels of biomarkers were compared between cases and controls using the Mann-Whitney *U* test given that the levels of these markers were not normally distributed and that log-transformation did not improve the normality of these variables. Moreover, we evaluated the associations between characteristics of study subjects and levels of sRAGE and VEGF using χ^2 test in order to identify potential confounding factors for these two biomarkers because sRAGE and VEGF were shown to be associated with risk of colorectal adenoma in the association analysis.

Odds ratio (OR) and its associated 95% confidence interval (95% CI) of colorectal adenoma according to each biomarker was estimated using unconditional logistic regression models. The levels of sRAGE, sTNF-αRI, sTNF-αRII, sIL-6R, EGF, and MCP1 were categorized based on the tertile distributions among controls, with the lowest tertile serving as the reference cat-

Table 1. Selected characteristics of cases with colorectal adenoma and polyp-free controls

Characteristics	Cases (n = 158)	Controls (n = 203)	*P* value
Age at colonoscopy (years)			0.09
Mean	61.0	62.4	
50-60	27.8	38.9	
60-70	58.9	49.3	
>70	13.3	11.8	
Race (%)			0.90
Non-Hispanic White	48.1	49.8	
African -American	38.6	38.4	
Other	13.3	11.8	
Sex (%)			0.41
Men	98.7	97.5	
Women	1.3	2.5	
Tobacco smoking status (%)			0.13
Never	19.6	28.1	
Former	54.4	45.3	
Current	26.0	26.6	
Alcohol drinking (%)			0.17
Never	5.8	6.9	
Former	43.0	33.2	
Current	51.2	59.9	
Physical activity (%)			0.04
Low	82.0	71.0	
Medium	8.7	17.9	
High	9.3	11.1	
Type 2 diabetes (%)	59.5	53.2	0.23
Hypertension (%)	57.0	49.3	0.14
NSAID use in the past 3 months (%)	50.0	56.7	0.20
BMI (Kg/m^2,mean ± SD)	30.8 ± 6.3	30.6 ± 6.0	0.83
Waist to hip ratio (mean ± SD)	0.98 ± 0.06	0.96 ± 0.06	0.007
Serum creatinine (mg/dl, mean ± SD)	1.03 ± 0.01	0.99 ± 0.01	0.05

Abbreviations: body mass index (BMI), nonsteroidal anti-inflammatory agents (NSAIDs), standard deviation (SD).

egory. For VEGF, subjects with values below the detection limit formed the reference category, and the remaining subjects were categorized into two groups using the median levels among the controls whose levels were above the detection limit. Potential confounding factors included NSAIDs use (use three or more times per week during the past 3 months), smoking status (never, former and current), body mass index (BMI) (< 30 versus ≥ 30 kg/m^2), waist to hip ratio (WHR), physical activity (low, moderate, and high), type 2 diabetes (yes versus no), hypertension (yes versus no), and serum creatinine levels. Covariates were retained in the final model if the β coefficient for any of the main exposure variables (i.e. cytokine levels) changed by >10% when the covariates were removed. To account for any residual confounding effect of age, we included age at colonoscopy in all the multivariate models.

We used multinomial logistic regression models to examine the risk estimates according to the size of the adenoma (<5 mm or ≥5 mm in diameter) and the presence of multiple adenomas (single or multiple adenomas), adjusting for the same confounding factors as in the main analysis. In the multinomial model, the outcome variables had three categories, including non-polyp controls, small size, and large size adenoma or non-polyp controls, single, and multiple adenomas.

Given that sRAGE and other inflammatory biomarkers have been linked to hypertension, diabetes, and use of NSAIDs, the association analyses were stratified by these variables. In a sensitivity analysis, we excluded the samples on one plate with the intra-plate CV of greater than 25% for sRAGE, which indicated an imprecise measurement. Lastly, Pearson's correlation coefficient was used to evaluate the correlation between sRAGE levels in 11 samples measured using both multiplex assay and the Quantikine ELISA assay. All statistical tests were two sided, with an α level of 0.05 indicat-

Table 2. Distributions of plasma levels of biomarkers among cases with colorectal adenoma and polyp-free controls

Biomarkers (pg/ml)	Cases (n = 158)	Controls (n = 203)	*P**
	Median (intertertile range)	Median (intertile range)	
sRAGE	42.3 (25.5-102.2)	49.7 (27.2-100.5)	0.26
sTNFRI	1437 (899-2117)	1297 (859-2021)	0.26
sTNFRII	6352 (4445-8951)	6456 (4349-9201)	0.89
sIL6R	19036 (12705-29157)	18489 (12853-27574)	0.60
EGF	109 (48-277)	104 (41-219)	0.15
MCP1	405 (263-523)	400 (269-528)	0.95
VEGF†	69.3 (33.1-161)	52.4 (28.4-138)	0.06

Abbreviations: epidermal growth factor (EGF), monocyte chemotactic protein-1 (MCP1), soluble interleukin-6 receptor (sIL-6R), soluble receptor for advanced glycation end-products (sRAGE), soluble tumor necrosis factor receptor I (sTNFRI), soluble tumor necrosis factor receptor II (sTNFRII), vascular endothelial growth factor (VEGF). **P* value for Mann-Whitney U test. †Calculated from the values above the detection limit.

ing statistical significance. All analyses were performed using Stata version 12.0 (STATA incorporation, College Station, TX).

Results

Among 158 cases, 37% had multiple adenomas, 61% had small adenomas (< 5 mm in size), 85% of adenomas were tubular, 14% were tubulovillous (including 1 villous adenoma) and 1% was serrated.

Table 1 shows that the distributions of age, ethnicity and sex were not significantly different between 158 cases and 203 controls. Controls had more moderate and higher levels of physical activity than cases did (*P* = 0.04). Although cases and controls had equivalent BMI, the cases and higher WHR than controls (*P* = 0.001). Cases had borderline statistically significant higher serum levels of creatinine than controls (*P* = 0.05). There were no significant differences between cases and controls in the distribution of hypertension, diabetes, and smoking status.

The intra-plate CV was less than 20% for sTNFR1, sTNFRII, sIL6R, EGF, and MCP1. One out of 13 plates of the sRAGE assay had the intra-plate CV of 25%. We excluded the 28 samples on this plate in the sensitivity analysis for sRAGE. VEGF was undetectable for 35% study subjects and was examined using the undetectable group as the reference group. Most samples (70%) had undetectable TNFβ while the intra-plate CV was higher than 25% for IFNα2 and G-CSF for more than 50% of the plates, and therefore these three markers were not further evaluated in the association analysis. All inter-plate CVs for sRAGE, sTNFR1, sTNFRII, sIL6R, EGF, MCP1, and VEGF were less than 25%. Among controls, there were significant correlations between sTNFR1 and sTNFRII (r = 0.58); sRAGE with each of sTNFR1 (r = 0.31) and sTNFRII (r = 0.25); and EGF with VEGF (r = 0.32). However, in a multiple linear regression analysis, there were no significant independent determinants of any biomarkers when age, hypertension and diabetes were included in the models.

Table 2 presents the distributions of sRAGE, sTNFRI, sTNFRII, sIL6R, EGF, MCP1, and VEGF among cases and controls. The median levels of sRAGE were higher in controls than in cases. The levels of sTNFRI, EGF, and VEGF were higher in cases than in controls. However, the noted differences were not statistically significant.

The distributions of sRAGE and VEGF were also examined according to potential confounders. We found that higher sRAGE levels among the study subjects with hypertension (73.4 versus 53.6 pg/ml, *P* < 0.0001, Mann-Whitney test) and among current NSAIDs user (73.3 versus 53.1, *P* = 0.0001, Mann-Whitney test) than those who did not have these factors, respectively. However, this trend was not significant among controls. Plasma levels of VEGF were not correlated with demographic or exposure variables (data not shown).

Table 3 shows that when the highest was compared with the lowest tertile of sRAGE, the unadjusted OR (95% CI) of colorectal adenoma was 0.75 (0.45-1.24). Adjusting for smoking

Table 3. Associations between plasma levels of selected biomarkers and risk of colorectal adenomas

Tertile of biomarker*	Cases (n) /control (n)†	Crude OR (95% CI)	Adjusted OR‡ (95% CI)
sRAGE (pg/ml)			
<36.3	63/67	1.00	1.00
36.3-71.3	47/67	0.75 (0.45-1.24)	0.64 (0.38-1.09)
>71.3	46/67	0.73 (0.43-1.21)	0.55 (0.31-0.96)
P trend		0.22	0.03
sTNFRI (pg/ml)			
<1131	48/67	1.00	1.00
1131-1567	52/68	1.07 (0.64-1.79)	0.87 (0.50-1.53)
>1167	56/66	1.18 (0.71-1.98)	0.83 (0.46-1.51)
P trend		0.52	0.56
EGF(pg/ml)			
<65.5	41/66	1.00	1.00
65.5-141.7	47/66	1.15 (0.67-1.97)	1.03 (0.59-1.82)
>141.7	65/68	1.54 (0.92-2.58)	1.51 (0.88-2.59)
P trend		0.10	0.12
VEGF (pg/ml)			
Undetectable	64/93	1.00	1.00
<52.4	37/55	0.98 (0.59-1.65)	0.90 (0.52-1.56)
≥ 52.4	57/55	1.51 (0.92-2.45)	1.75 (1.05-2.93)
P trend		0.12	0.04

Abbreviations: confidence interval (CI), epidermal growth factor (EGF), odds ratio (OR), soluble receptor for advanced glycation end-products (sRAGE), soluble tumor necrosis factor receptor I (sTNFRI), vascular endothelial growth factor (VEGF). *Tertile was generated according to the distribution among controls. †The number of cases and controls do not add up to the total because of exclusion of outliers. ‡OR was adjusted for age, smoking status, hypertension and diabetes.

status, hypertension, and diabetes each strengthened the inverse association and adjustment for all three jointly contributed to a significant inverse association between sRAGE and risk of colorectal adenoma (OR = 0.55, 95% CI 0.31-0.96, *P* trend = 0.03). Compared with the undetectable levels, highest levels of VEGF were associated with an increased risk of colorectal adenoma (OR = 1.75, 95% CI 1.05-2.93, *P* trend = 0.04). Adjustments for current use of NSAIDs, WHR, physical activity, and creatinine levels did not change the parameter estimate by more than 10% for either association. The association between EGF levels and risk of colorectal adenoma did not reach statistical significance. There were no significant associations between sTNFRI, sTNFRII, sIL-6R, and MCP1 and the risk of colorectal adenoma.

In the multinomial logistic regression analyses (**Table 4**), we observed an inverse association between sRAGE and small size adenoma and single adenoma. The positive association between VEGF and risk of adenoma was more evident in those with small size adenomas. **Table 5** shows that the association between sRAGE and risk of colorectal adenoma differed by hypertension status where the strong inverse association was seen among study subjects without hypertension, but not among the study subjects with hypertension (*P* interaction = 0.02). Moreover, among study subjects without hypertension and diabetes (44 cases and 64 controls), sRAGE levels were associated with highly significant reduced risk of colorectal adenoma (OR = 0.17, 95% CI 0.06-0.52, *P* trend < 0.0001). The association between sRAGE or VEGF and risk of colorectal adenoma did not differ by type 2 diabetes or NSAIDs use.

For sRAGE, we excluded 28 samples on one plate with high intra-plate CV in the sensitivity analysis. The OR of colorectal adenoma was 0.53 (95% CI 0.30-0.94) for the 2nd tertile and 0.51 (95% CI 0.28-0.91) for the highest tertile (*P* trend = 0.02). The correlation study using 11 samples found a significant positive correlation between the sRAGE levels measured by the multiplex assay and the Quantikine ELISA (r = 0.71, *P* = 0.001).

Discussion

This case-control study supports the involvement of RAGE related inflammatory mechanism in the development of colorectal adenoma. We found that high sRAGE was inversely associated, while high serum VEGF levels were positive-

Table 4. Associations between plasma levels of sRAGE and VEGF and colorectal adenomas according to size and number of adenoma

Features of adenoma	sRAGE levels*			VEGF levels*		
Size < 5 mm	Tertile 1	Tertile 2	Tertile 3	Tertile 1	Tertile 2	Tertile 3
Case/control†	36/67	24/67	25/67	32/93	20/55	34/55
Crude OR (95% CI)	1.00	0.68 (0.37-1.22)	0.73 (0.41-1.32)	1.00	1.04 (0.57-1.91)	1.79 (1.02-3.08)
Adjusted OR‡ (95% CI)	1.00	0.59 (0.32-1.08)	0.59 (0.32-1.10)	1.00	1.00 (0.53-1.88)	2.01 (1.14-3.57)
Size ≥ 5 mm						
Case/control†	24/67	18/67	13/67	25/93	13/55	16/55
Crude OR (95% CI)	1.00	0.82 (0.40-1.68)	0.55 (0.25-1.22)	1.00	0.88 (0.42-1.86)	1.08 (0.53-2.20)
Adjusted OR‡ (95% CI)	1.00	0.74 (0.35-1.54)	0.46 (0.20-1.05)	1.00	0.86 (0.40-1.84)	1.11 (0.54-2.30)
Single						
Case/control†	44/67	21/67	28/67	38/93	24/55	33/55
Crude OR (95% CI)	1.00	0.46 (0.24-0.85)	0.57 (0.32-1.04)	1.00	1.07 (0.58-1.97)	1.47 (0.83-2.60)
Adjusted OR‡ (95% CI)	1.00	0.41 (0.22-0.78)	0.48 (0.25-0.90)	1.00	1.03 (0.55-1.92)	1.57 (0.86-2.83)
Multiple						
Case/control†	19/67	26/67	18/67	26/93	13/55	24/55
Crude OR (95% CI)	1.00	1.38 (0.69-2.76)	0.92 (0.43-1.94)	1.00	0.85 (0.40-1.78)	1.56 (0.82-2.98)
Adjusted OR‡ (95% CI)	1.00	1.21 (0.59-2.47)	0.72 (0.32-1.58)	1.00	0.82 (0.38-1.77)	1.76 (0.90-3.45)

Abbreviations: confidence interval (CI), odds ratio (OR), soluble receptor for advanced glycation end-products (sRAGE), vascular endothelial growth factor (VEGF). *Tertile was generated according to the distribution among the controls in each stratum. †The number of cases and controls do not add up to the total because of exclusion of outliers. ‡OR was adjusted for age, smoking status, hypertension and diabetes.

ly associated with an increased risk of having colorectal adenomas. The inverse association between sRAGE and risk of colorectal adenoma differed by hypertension status and was seen only among patients without hypertension. There were no statistically significant associations for sTNFR1, sTNFRII, sIL-6R, EGF, and MCP1.

Our study was consistent with our previous finding of an inverse association between sRAGE and risk of colorectal cancer [11]. The association was unrelated to the size and multiplicity of adenomas. It suggested that the RAGE-related mechanism is involved in the early development of colorectal adenomas. RAGE expression, especially with membranous pattern, has been associated with malignant potential of colorectal adenomas in human in an immunohistochemistry study of 35 of 96 adenomas [13]. RAGE, by combining with HMGB1, can activate NF-κB and induce the upregulation of leukocyte adhesion molecules and the production of proinflammatory cytokines and angiogenic factors in both hematopoietic and endothelial cells. Chronic stimulation of RAGE perpetuates the inflammatory response generated in response to a different initiating event. Interestingly, ligands of RAGE, such as HMGB1, may also signal through toll-like receptors (TLRs), TLR2 and TLR4, to promote inflammation [14] partially through activation of the NF-κB pathway. TLR2 and TLR4 have been implicated in the development of colorectal cancer as well [15, 16].

Although our two independent studies in colorectal cancer and adenoma both observed an inverse association between sRAGE and colorectal neoplasms, the antibody used in the Quantikine ELISA and the multiplex assay may be different because the absolute levels of the sRAGE analytes were not on the same scale for these two assays. Nevertheless, our study found a significant positive correlation between the sRAGE levels measured by these two assays. It indicates that similar antigen moiety may have been captured by two assays. The consistent findings from these two studies jointly indicated the involvement of RAGE in colorectal tumorigenesis.

It is not entirely clear why we did not observe an association of sRAGE with risk of colorectal adenoma among patients with hypertension. It is possible that the use of anti-hypertension medications have masked such an association. It was reported that long-term treatment with a combination of nifedipine-telmisartan may increase sRAGE plasma levels, thus exerting an atheroprotective and anti-inflammatory activity [17]. The interrelation among hypertension medications, sRAGE and colorectal adenoma deserves further investigation in considering sRAGE can be modulated by anti-hypertension medications.

Table 5. Associations between plasma levels of sRAGE and VEGF and colorectal adenomas by hypertension, diabetes and use of NSAIDs

Stratum	sRAGE levels*			VEGF levels*		
Hypertension - No	Tertile 1	Tertile 2	Tertile 3	Tertile 1	Tertile 2	Tertile 3
Case/control†	39/32	16/35	13/34	33/51	11/26	24/26
Crude OR (95% CI)	1.00	0.37 (0.18-0.80)	0.31 (0.14-0.69)	1.00	0.65 (0.29-1.50)	1.43 (0.70-2.89)
Adjusted OR‡ (95% CI)	1.00	0.33 (0.15-0.73)	0.22 (0.09-0.53)	1.00	0.67 (0.28-1.60)	1.56 (0.75-3.24)
Hypertension - Yes						
Case/control†	26/32	30/34	32/34	31/42	23/29	36/29
Crude OR (95% CI)	1.00	1.08 (0.53-2.21)	1.16 (0.57-2.35)	1.00	1.07 (0.52-2.20)	1.68 (0.86-3.30)
Adjusted OR‡ (95% CI)	1.00	1.07 (0.51-2.26)	1.03 (0.49-2.17)	1.00	1.05 (0.50-2.22)	2.04 (0.99-4.17)
Diabetes - No						
Case/control†	44/40	25/41	30/41	41/54	24/34	35/35
Crude OR (95% CI)	1.00	0.55 (0.29-1.07)	0.67 (0.35-1.26)	1.00	0.93 (0.48-1.80)	1.32 (0.71-2.45)
Adjusted OR‡ (95% CI)	1.00	0.51 (0.26-1.00)	0.59 (0.30-1.16)	1.00	0.88 (0.44-1.76)	1.40 (0.74-2.62)
Diabetes - Yes						
Case/control†	17/22	24/24	12/23	21/35	8/17	25/17
Crude OR (95% CI)	1.00	1.29 (0.55-3.02)	0.68 (0.26-1.73)	1.00	0.78 (0.29-2.13)	2.45 (1.08-5.56)
Adjusted OR‡ (95% CI)	1.00	0.97 90.39-2.41)	0.44 (0.16-1.25)	1.00	0.77 (0.28-2.18)	2.63 (1.10-6.29)
No NSAIDs use						
Case/control†	33/28	24/29	20/30	28/40	18/24	33/24
Crude OR (95% CI)	1.00	0.70 (0.34-1.47)	0.56 (0.26-1.21)	1.00	1.07 (0.49-2.33)	1.96 (0.96-4.01)
Adjusted OR‡ (95% CI)	1.00	0.60 (0.28-1.30)	0.42 (0.19-0.95)	1.00	1.02 (0.45-2.34)	2.03 (0.96-4.28)
NSAIDs use						
Case/control†	29/36	24/39	26/39	36/53	16/31	27/31
Crude OR (95% CI)	1.00	0.76 (0.38-1.55)	0.83 (0.41-1.66)	1.00	0.76 (0.36-1.59)	1.28 (0.66-2.50)
Adjusted OR‡ (95% CI)	1.00	0.75 (0.35-1.60)	0.57 (0.26-1.24)	1.00	0.75 (0.35-1.63)	1.54 (0.75-3.18)

Abbreviations: confidence interval (CI), odds ratio (OR), nonsteroidal anti-inflammatory agents (NSAIDs), soluble receptor for advanced glycation end-products (sRAGE), vascular endothelial growth factor (VEGF). *Tertile was generated according to the distribution among the controls in each stratum. †The number of cases and controls do not add up to the total because of exclusion of outliers. ‡OR adjusted for age, smoking status, hypertension and/or diabetes.

RAGE signaling can activate both VEGF and EGF through NF-κB pathway. In colorectal cancer cells, RAGE knockdown inhibited VEGF expression [18]. Inhibition of VEGF and EGF signaling reduced both the number and the size of polyps at either an early or a later stage of polyp development in the Apc(Min/+) mouse [19]. Whereas VEGF has been related to lymph node metastasis in colorectal carcinoma, its role in colorectal adenoma is less clear. In a human study, colonic adenomas showed a statistically significant up-regulation of VEGF expression in 12 adenomas compared with 11 normal colon tissue specimens, with a further increase during the development of adenocarcinomas [20]. Three other human studies also showed that the members of VEGF receptors, VEGF-A, B, C were more abundantly detected in colorectal adenoma than in normal colon tissues [21-23]. Serum VEGF levels were examined in one Japanese study that found a significantly higher levels of VEGF in 67 patients with colorectal cancer than in 14 patients with colorectal adenomas or 72 normal controls [24]. Similar to our observation, these studies suggest that angiogenic switch VEGF may be turned on during the formation of colorectal adenoma.

We did not observe a statistically significant increased risk of adenoma in association with EGF. The limited studies on EGF and EGFR and colorectal adenoma were inconsistent with some showing that EGF expression was not elevated in adenomas or in early stage of colorectal tumorigenesis [25, 26], and the other study showing that EGF receptor-associated tyrosine kinase plays an important role in the development of hyperproliferative state of the colonic mucosa and colon carcinogenesis [27]. More studies are needed to further elucidate the role of EGF/EGFR in colorectal tumorigenesis.

Our study has some limitations and also several strengths. Firstly, the study was based on small sample size because we applied strict exclusion criteria in selecting study subjects to avoid outcome misclassification. The findings

would be insignificant if *P* value is corrected for multiple testing. Secondly, although the advantage of simultaneous quantification of multiple markers is multifaceted, including specimen conservation, reduced sample handling and thaw-freeze cycle, and less labor and time, the inaccurate lab assay may have had affected the results. For instance, we had to abandon the measurement values for IFNα2, G-CSF, and TNFβ. Nevertheless, our robust quality control measures helped with the interpretation of the study findings and avoided the spurious association that might have generated due to the imprecise measurements. Because we placed the matched cases and controls on the same plate, the misclassification of the biomarkers to tertiles was non-differential, and therefore was likely to drive the association towards the null. Thirdly, the study was limited by the fact that we did not measure the gene expression levels in colon tissue. Therefore, the direct relevance of the cytokines in the formation of colorectal adenoma cannot be established. For example, the expression of MCP1 in colorectal adenoma epithelial cells of human tissue samples was shown to involve in macrophage migration and COX–2 expression, which leads to the subsequent development of colonic adenoma [28]. However, we did not observe the difference of plasma levels of MCP1 between cases and controls in our study. Lastly, our study findings may not be generalized to women because the majority of the study subjects were men.

In summary, in conjunction with our previous research [11], this study supports the involvement of chronic information in particular the RAGE-related mechanism in the development of colorectal adenomas. In addition, we showed that VEGF may also be involved in the development of colorectal adenomas. Our findings lend further evidence that systemic inflammation plays a role in the early development of colorectal neoplasia [29]. Previous research has shown that antioxidant intake may interact with inflammation in modifying the recurrence of advanced adenoma [30] and antioxidant micronutrients can modulate the oxidative and inflammatory biomarkers in patients with a history of sporadic colorectal adenoma [31, 32]. Therefore, further understanding on biomarkers of tumor microenvironment, dietary intake, and their interaction could help identify preventive strategy for colorectal neoplasia. The RAGE-NF-κB related mechanism may be one of these targets.

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Disclosure statement

The authors have no conflicts of interest to disclose.

Disclaimer

The views expressed in this article are those of the authors and do not necessarily reflect the position or policy of the Department of Veterans Affairs or the United States government.

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