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*Cancer Res* 2011;71:3582-3589. Published OnlineFirst May 3, 2011.

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## Evidence That Serum Levels of the Soluble Receptor for Advanced Glycation End Products Are Inversely Associated with Pancreatic Cancer Risk: A Prospective Study

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### Abstract

Cigarette smoking, obesity, type 2 diabetes, and, to a lesser extent, meat cooked at high temperatures are associated with pancreatic cancer. Cigarette smoke and foods cooked at higher temperatures are major environmental sources of advanced glycation end products (AGE). AGEs accumulate during hyperglycemia and elicit oxidative stress and inflammation through interaction with the receptor for AGEs (RAGE). Soluble RAGE (sRAGE) acts as an anti-inflammatory factor to neutralize AGEs and block the effects mediated by RAGE. In this study, we investigated the associations of prediagnostic measures of N<sup>ε</sup>-(carboxymethyl)-lysine (CML)-AGE and sRAGE with pancreatic cancer in a case-cohort study within a cohort of 29,133 Finnish male smokers. Serum samples and exposure information were collected at baseline (1985–1988). We measured CML-AGE, sRAGE, glucose, and insulin concentrations in fasting serum from 255 incident pancreatic cancer cases that arose through April 2005 and from 485 randomly sampled subcohort participants. Weighted Cox proportional hazard regression models were used to calculate relative risks (RR) and 95% CI, adjusted for age, years of smoking, and body mass index. CML-AGE and sRAGE were mutually adjusted. CML-AGE levels were not associated with pancreatic cancer [fifth compared with first quintile, RR (95% CI): 0.68 (0.38–1.22),  $P_{\text{trend}} = 0.27$ ]. In contrast, sRAGE levels were inversely associated with pancreatic cancer [fifth compared with first quintile, RR (95% CI): 0.46 (0.23–0.73),  $P_{\text{trend}} = 0.002$ ]. Further adjustment for glucose or insulin levels did not change the observed associations. Our findings suggest that sRAGE is inversely associated with pancreatic cancer risk among Finnish male smokers. *Cancer Res*; 71(10); 3582–9. ©2011 AACR.

### Introduction

Cigarette smoking, obesity, and type 2 diabetes are established risk factors of pancreatic cancer (1). Although less consistent, intakes of red meat and high-fat processed foods, particularly from animal sources, have also been associated with pancreatic cancer (2, 3). Insulin resistance, inflammation, and oxidative stress are the hypothetical mechanisms underlying the etiology of pancreatic cancer (4, 5). Advanced glycation end products (AGE) and receptor for advanced glycation end products (RAGE) contribute to the development of insulin resistance, inflammation, and oxidative stress (6, 7). However,

it is unknown whether the AGE–RAGE axis has any implication in pancreatic cancer etiology.

AGEs are a heterogeneous group of irreversible adducts formed by the addition of an aldehyde or ketone group from reducing sugars to an amino group on lipids, proteins, or nucleic acids through glycation (8). AGEs can form endogenously through normal metabolism and exogenously from environmental exposure. Commonly consumed foods with high content of AGEs (e.g., French fries, broiled beef, fried chicken, and American cheese; ref. 9) and tobacco smoke represent the major environmental sources of AGEs to which the general public is exposed (10–12). AGEs accumulate in human tissue proteins with advancing age, and this accumulation accelerates in the presence of hyperglycemia (13). N<sup>ε</sup>-(carboxymethyl)-lysine (CML)-AGE is one of the best characterized AGEs. CML-AGE is a biomarker of long-lasting oxidative stress resulting from carbohydrate and lipid oxidation reactions (14).

Cell membrane-bound full-length RAGE is a multi-ligand receptor and was first described as a signal transduction receptor for AGEs (15). The engagement of RAGE with AGEs elicits oxidative stress and evokes inflammatory and thrombotic responses (16). Full-length RAGE has been described as a link between chronic inflammation and cancer (17). Soluble form of RAGE (sRAGE) is abundant in the circulation

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doi: 10.1158/0008-5472.CAN-10-2573

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in humans. sRAGE has AGEs-binding properties but in the absence of intracellular domain that is essential for RAGE signaling. Therefore, sRAGE may neutralize circulating AGEs and protect against RAGE-mediated cascades (18).

Several clinical and experimental studies have sparked research interests in AGEs/RAGE and carcinogenesis (19–22). Three hospital-based studies have shown lower levels of sRAGE in patients with cancers of breast (23), lung (24), and pancreas (25) compared with healthy controls; however, the AGEs–RAGE axis has not been examined in relation to cancer risk in a prospective epidemiologic study. We, therefore, investigated the associations of serum levels of CML-AGE and sRAGE with pancreatic cancer risk in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study (26). We hypothesized that higher levels of CML-AGE or lower levels of sRAGE are associated with a greater risk of pancreatic cancer. Because type 2 diabetes and impaired glucose tolerance are risk factors for pancreatic cancer and AGEs accumulate in human tissue proteins as a result of chronic hyperglycemia, we also explored the influence of serum glucose on the association of CML-AGE or sRAGE with pancreatic cancer.

## Materials and Methods

### Study population

This study was a case–cohort study (27) within the ATBC Study. The ATBC Study was a randomized, double-blinded, placebo-controlled,  $2 \times 2$  factorial design, primary prevention trial conducted in southwest Finland that tested whether  $\alpha$ -tocopherol and/or  $\beta$ -carotene supplement could reduce the incidence of lung cancer and other major cancers among male smokers (26). Potential participants were excluded from the trial for the following reasons: malignancy other than nonmelanoma skin cancer or carcinoma *in situ*; severe angina on exertion; chronic renal insufficiency; cirrhosis of liver; chronic alcoholism; receiving anticoagulant therapy; other medical problems that might limit participation for 6 years; and current use of supplements containing vitamin E, vitamin A, or  $\beta$ -carotene. Between 1985 and 1988, 29,133 eligible men in the age range 50 to 69 years who smoked at least 5 cigarettes per day were randomized to receive an active supplement or a placebo. The trial ended in April 1993 but participants continue to be followed for health outcomes through national registries. The ATBC Study was approved by the institutional review groups of the US National Cancer Institute and the National Public Health Institute of Finland (now National Institute for Health and Welfare, Helsinki, Finland). All participants provided written informed consent before randomization.

To reduce the potential influence of subclinical pancreatic cancer on serum CML-AGE or sRAGE levels (i.e., reverse causality), all eligible study subjects were cohort members who were alive without a cancer diagnosis as of the sixth year of follow-up. Therefore, the follow-up started after 5 years of baseline and ended at death, diagnosis of pancreatic cancer, or on April 30, 2005. From a total of 24,708 eligible study participants, we identified 260 incident cases of primary

pancreatic cancer [International Classification of Diseases (ICD)-9 157, excluding 157.4] from the Finnish Cancer Registry (28). Five hundred subcohort participants were randomly selected from all eligible study subjects as the reference group. Four hundred of them served as the reference group in a previous study on insulin resistance and pancreatic cancer risk (5). The present analysis included 255 cases and 485 subcohort participants after we excluded 5 cases and 15 subcohort participants whose data on any of the 4 serologic biomarkers were missing due to an empty vial.

### Data collection and serologic assay

At the baseline visit and prior to randomization, all participants completed a self-administered questionnaire to provide information on general demographics, medical, smoking, and occupational histories as well as dietary intake during the past year (using a validated self-administered dietary history questionnaire). Height and weight were measured by trained nurses. Body mass index (BMI) was calculated as (weight in kilograms)/(height in meters squared) (26, 29). Study participants provided a venous blood sample after an overnight fast. Serum was isolated, aliquoted, and stored in the dark at  $-70^{\circ}\text{C}$ . The freeze-thaw cycle of the serum samples was documented and counted. Eighty-one percent of sera had not undergone more than 2 freeze-thaw cycles before this experiment.

CML-AGE and sRAGE levels were measured in duplicate at the Microcoat Biotechnologie Company by using an AGE-CML-ELISA kit (Microcoat Biotechnologie Company) and a human sRAGE Quantikine ELISA kit (R&D system Inc.), respectively. The AGE-CML-ELISA kit uses a CML-specific monoclonal antibody (mouse monoclonal 4G9; Alteon Inc.) and shows no cross-reactivity with un-derivatized lysine, glycine, or alanine (30). The sRAGE Quantikine kit detects a heterogeneous group of total sRAGE proteins, including cleavage forms of membrane-bound full-length RAGE, endogenous secretory RAGE (esRAGE), and other splice variant forms of RAGE. Case and subcohort samples (in  $\sim 1:2$  ratio) were randomly ordered in each batch and the laboratory personnel were blinded to case and subcohort status. To assess the reliability of the ELISA assays, we included 10% blinded, phantom, quality control (QC) samples from a single pooled serum sample in each batch.

The measurement of serum concentrations of glucose and insulin for 167 cases and 400 subcohort participants was described in detail previously (5). In this study, we measured insulin and glucose concentrations on an additional 93 cases that occurred after 2001 and on 100 additional subcohort participants, using the same laboratory as the earlier study. Our pilot study using 19 previously measured samples showed a good concordance of the measurements done at the different time points.

### Statistical analysis

We compared the levels of CML-AGE, sRAGE, and the CML-AGE/sRAGE ratio between the cases and the subcohort by the Wilcoxon rank-sum test. In the subcohort, the correlations between the biomarkers and selected exposure variables were

examined by partial Spearman's rank correlation coefficients. Dietary intake variables were adjusted for total energy intake by the residual method (31). The coefficients of variation (CV) and the intra-class correlation coefficient (ICC) were calculated by using the data on the QC samples. The CV was for the total variability of the sum of the between-batch and within-batch variances. The ICC was based on a random-effects model with a random batch effect and within-batch random error effect.

To estimate CML-AGE- and sRAGE-specific relative risks (RR) of pancreatic cancer, compute 95% CI, and perform significance tests for trends and interactions, we used weighted Cox proportional hazard regression models. The quintile cutoff points were generated according to the distribution in the subcohort, and the lowest quintile was the reference category. The weighted analysis was done to account for the differential sample rates of the cases and the subcohort participants in the survival analysis where each subject's weight was the inverse of their sample fraction. Cases were given a sample weight of 1 because they were sampled with certainty, whereas subcohort participants were given a sample weight of 49.4 (24,708/500; ref. 27). We used the follow-up time as the underlying time metric. The assumption of proportional hazard was tested by generating the interaction term of each exposure variable and person-year of follow-up. Because CML-AGE and sRAGE were correlated and sRAGE is thought to neutralize unbound CML-AGE, we calculated the CML-AGE/sRAGE ratio and evaluated this ratio in relation to pancreatic cancer risk. Linear trends were tested by a score variable based on the median value of each quintile. On the basis of the univariate analysis, age, BMI, the number of years of smoking, total energy intake, daily average intakes of total and saturated fat, red meat, available carbohydrates, carbohydrate nutrients, and alcohol use (all on a continuous scale) were evaluated as potential confounders. A variable was defined as a confounding factor if it was significantly associated with pancreatic cancer risk and CML-AGE or sRAGE, or its addition in the model changed the risk estimate for CML-AGE or sRAGE by more than 10%. Although none of the variables was a confounder, we included age at randomization, years of smoking, and BMI in all models because they are putative risk factors for pancreatic cancer. To assess whether the effects of CML-AGE or sRAGE on pancreatic cancer risk were independent of each other and of glucose and insulin, we examined the change of risk estimates by adding the other 3 variables in the model one at a time (on a continuous scale). The continuous variables with a skewed distribution were log-transformed prior to further analyses.

Stratified analyses were used to evaluate interactions between glucose (dichotomous) and CML-AGE and sRAGE (in quintiles on pancreatic cancer risk). Because glucose levels assayed at 2 time points had slightly different distributions, the time-specific medians among the subcohort participants were used to dichotomize glucose levels with 99 mg/dL for the earlier time point and 77 mg/dL for the present time point. Interactions were represented as cross-product terms and the statistical significance of an interaction was tested by the Wald test. Additional stratified analyses were done according

to serum levels of insulin, the number of years of smoking (using the median among the subcohort participants as the cutoff point), BMI (<25 vs.  $\geq$ 25), and intervention groups (placebo,  $\alpha$ -tocopherol,  $\beta$ -carotene, or both). In addition, we examined the joint effects (i.e., the departure from a multiplicative model of interaction) of CML-AGE and sRAGE (both were dichotomous) on the risk of pancreatic cancer by a  $2 \times 4$  table.

To evaluate whether using the insulin and glucose levels measured at 2 time points affected our results, we limited all the analyses to the 167 cases and 400 subcohort participants whose measurements were done in the earlier study. Furthermore, sensitivity analyses were done by excluding participants with self-reported diabetes ( $n = 33$ ) or participants who died or were censored in the first 10 or 15 years of follow-up, respectively. An additional sensitivity test was conducted by excluding the subjects whose sera had undergone more than 2 freeze-thaw cycles with adjustment for freeze-thaw cycle in the model. All tests were 2-sided and  $P \leq 0.05$  indicated statistical significance. SAS 9.0 (SAS institute, Inc.) and SUDAAN software (Research Triangle Park) were used for data analyses.

## Results

The interval between serum collection and pancreatic cancer diagnosis was up to 20 years, with a median of 15 years. Selected characteristics of 255 cases and 485 subcohort participants are described in Table 1. The mean value of the duplicate of CML-AGE and sRAGE measurements was used in

**Table 1.** Baseline characteristics of pancreatic cancer cases and subcohort participants in the ATBC Study, 1988–2005

Characteristics	Cases ( $n = 255$ )	Subcohort ( $n = 485$ )
Demography		
Age, years	57.6 (4.9)	56.4 (5.0)
BMI, kg/m <sup>2</sup>	26.4 (3.5)	26.7 (3.8)
Years of smoking	36.2 (7.6)	35.2 (7.7)
History of diabetes mellitus, % yes	5.9	4.3
Dietary intake <sup>a</sup> , g/d		
Total fat	102 (13.6)	101 (15.2)
Protein	93.7 (12.2)	94.7 (12.7)
Red meat	72.8 (31.2)	68.3 (28.2)
Processed meat	70.7 (52.8)	74.5 (52.4)
Available carbohydrate	263.3 (36.8)	264.8 (39.1)
Glucose	9.32 (5.31)	9.30 (5.76)

NOTE: All participants were alive without cancer 5 years after baseline. Data are mean (SD) for the continuous variables and percentage for the categorical variables.

<sup>a</sup>Using residual method to adjust for total energy intake for food or nutrient variables.

**Table 2.** Partial Spearman correlation coefficient (*r*) for the correlation of selected characteristics with CML-AGE, sRAGE, and CML/sRAGE ratio in the subcohort in the ATBC Study

Characteristics	CML-AGE <sup>a</sup>	sRAGE <sup>a</sup>	CML/sRAGE ratio
Age at randomization <sup>b</sup>	-0.01	-0.009	0.02
BMI <sup>b</sup> , kg/m <sup>2</sup>	-0.09 <sup>d</sup>	-0.03	-0.01
Years of smoking <sup>b</sup>	-0.02	-0.03	0.02
Total fat, g/day	-0.10 <sup>d</sup>	-0.02	-0.004
Protein, g/day	-0.05	0.02	-0.007
Red meat, g/day	0.004	-0.005	0.006
Processed meat, g/day	0.02	-0.05	0.06
Available carbohydrate intake <sup>c</sup> , g/day	0.07	0.08	-0.07
Glucose intake <sup>c</sup> , g/day	0.11 <sup>d</sup>	-0.06	0.06
Alcohol consumption <sup>c</sup> , g/day	0.05	-0.09	0.08
Serum insulin <sup>a,c</sup>	-0.04	0.02	-0.02
Serum glucose <sup>a,c</sup>	0.07	-0.11 <sup>d</sup>	0.12 <sup>d</sup>
CML-AGE <sup>a,c</sup>		0.48 <sup>d</sup>	

<sup>a</sup>Log-transformed values were used.

<sup>b</sup>Coefficient values adjusted for age at randomization, BMI, or years of smoking.

<sup>c</sup>Coefficient values adjusted for age at randomization, BMI, years of smoking, sRAGE (for CML-AGE), or CML-AGE (for sRAGE).

<sup>d</sup> $P < 0.05$  for the correlation.

the analyses and the CV of the duplicates were all less than 20%. The median level (interquartile range) of CML-AGE was 533 (397–645) ng/mL for the cases and 561 (471–668) ng/mL for the subcohort. The median level (interquartile range) for sRAGE was 482 (344–632) pg/mL for the cases and 572 (417–742) pg/mL for the subcohort. Cases had significantly lower levels of CML-AGE and sRAGE than the subcohort participants did ( $P < 0.005$ ). The median level (interquartile range) of the CML-AGE/sRAGE ratio was 1,027 (779–1,425) for the cases and 998 (732–1,303) for the subcohorts ( $P = 0.06$ ). The CV and the ICC was 9.2% and 0.64 for CML-AGE and 5.7% and 0.78 for sRAGE, respectively.

Table 2 presents the partial Spearman correlation coefficient for the examined biomarkers and the selected characteristics in the subcohort, after adjusting for age, BMI, and the number of years of smoking. There was a significant, moderate, positive correlation between serum CML-AGE and sRAGE ( $P < 0.001$ ). CML-AGE had a weak negative correlation with BMI and total fat intake and had a positive correlation with daily glucose intake. sRAGE and CML-AGE/sRAGE ratio were negatively correlated with serum glucose, and to a lesser extent, alcohol consumption.

Table 3 shows the association among CML-AGE, sRAGE, and the CML-AGE/sRAGE ratio and risk of pancreatic cancer. Higher levels of CML-AGE tended to be inversely associated with pancreatic cancer risk in a threshold pattern (models 1 and 2). Adjustment for sRAGE attenuated this association (model 3). Higher levels of sRAGE were significantly associated with a reduced risk of pancreatic cancer in a dose–response manner [fifth compared with first quintile, RR (95% CI): 0.46 (0.23–0.73),  $P_{\text{trend}} = 0.002$ ] after adjustment for age at randomization, the number of years of smoking, BMI, and CML-AGE (model 3). Adjustment for serum glucose did not change the

risk estimates (model 4), nor did the adjustment for insulin (data not shown). Adjustment for freeze–thaw cycle of serum did not change our major finding. The RR (95% CI) related to sRAGE was 0.42 (0.20–0.86; fifth compared with first quintile,  $P_{\text{trend}} < 0.001$ ). A high CML-AGE/sRAGE ratio was associated with an increased risk of pancreatic cancer.

Table 4 shows that the effect modification of CML-AGE by sRAGE was on a sub-multiplicative scale ( $P$  interaction = 0.048). Compared with lower levels of CML-AGE and higher levels of sRAGE, the RR of the pancreatic cancer for the higher levels of CML-AGE and lower levels of sRAGE was 2.07 (95% CI: 1.17–3.67), which was less than the expected joint RR by the product of the individual effects of higher levels of CML-AGE [RR: 1.41 (95% CI: 0.82–2.45)] and lower levels of sRAGE [RR (95% CI): 3.00 (1.79–5.03)]. We examined the respective interactions of serum glucose with either CML-AGE or sRAGE on risk of pancreatic cancer. High serum CML-AGE was associated with an increased risk of pancreatic cancer among men who had higher levels of glucose [RR (95% CI): 2.72 (1.18–6.25); fifth compared with first quintile; 144 cases and 254 subcohort participants] and was associated with a reduced risk among men who had lower levels of glucose [RR: 0.52 (95% CI: 0.23–1.18), fifth compared with first quintile, 113 cases and 244 subcohort participants; data not shown]. However, neither this interaction nor that between sRAGE levels and glucose was significant ( $P$  values for interaction = 0.33 and 0.10, respectively). There was no evidence of CML-AGE or sRAGE–pancreatic cancer interactions with insulin, smoking, BMI, or the trial intervention ( $P$  values for interaction  $> 0.30$ ; data not shown).

These results remained the same when all the analyses were carried out among 167 cases and 400 subcohort participants, whose glucose and insulin concentrations were

**Table 3.** RR and 95% CI of pancreatic cancer according to quintiles of CML-AGE, sRAGE, and the CML-AGE/sRAGE ratio in the ATBC Study

	Quintiles					<i>P</i> <sub>trend</sub>
	1	2	3	4	5	
<b>CML-AGE</b>						
Range, ng/mL	<437	437–526	527–595	596–692	≥693	
Case/subcohort, <i>n/n</i>	83/97	41/97	42/97	48/97	41/97	
Model 1 <sup>a</sup> , RR (95% CI)	1.00	0.49 (0.30–0.80)	0.48 (0.29–0.79)	0.60 (0.37–0.97)	0.46 (0.28–0.75)	0.003
Model 2 <sup>b</sup> , RR (95% CI)	1.00	0.49 (0.30–0.80)	0.47 (0.29–0.78)	0.58 (0.35–0.95)	0.45 (0.27–0.73)	0.003
Model 3 <sup>c</sup> , RR (95% CI)	1.00	0.66 (0.38–1.16)	0.62 (0.36–1.06)	0.82 (0.47–1.43)	0.68 (0.38–1.22)	0.27
Model 4 <sup>d</sup> , RR (95% CI)	1.00	0.62 (0.35–1.08)	0.60 (0.35–1.03)	0.81 (0.47–1.42)	0.72 (0.39–1.30)	0.37
<b>sRAGE</b>						
Range, pg/mL	<374	374–502	503–630	631–793	≥794	
Case/subcohort, <i>n/n</i>	76/97	61/97	54/97	29/97	35/97	
Model 1 <sup>a</sup> , RR (95% CI)	1.00	0.75 (0.47–1.19)	0.69 (0.43–1.11)	0.36 (0.21–0.62)	0.39 (0.24–0.66)	0.0001
Model 2 <sup>b</sup> , RR (95% CI)	1.00	0.72 (0.44–1.16)	0.69 (0.42–1.12)	0.36 (0.21–0.63)	0.40 (0.24–0.67)	0.0002
Model 3 <sup>c</sup> , RR (95% CI)	1.00	0.81 (0.49–1.36)	0.77 (0.46–1.28)	0.41 (0.23–0.73)	0.46 (0.23–0.73)	0.002
Model 4 <sup>d</sup> , RR (95% CI)	1.00	0.77 (0.46–1.29)	0.72 (0.43–1.22)	0.38 (0.21–0.68)	0.43 (0.24–0.77)	0.0007
<b>CML-AGE/sRAGE ratio</b>						
Range	<672	672–871	872–1,108	1,109–1,398	≥1,399	
Case/subcohort, <i>n/n</i>	37/97	55/97	48/97	47/97	68/97	
Model 1 <sup>a</sup> , RR (95% CI)	1.00	1.45 (0.86–2.45)	1.30 (0.76–2.22)	1.38 (0.81–2.36)	2.02 (1.21–3.38)	0.01
Model 2 <sup>b</sup> , RR (95% CI)	1.00	1.46 (0.86–2.46)	1.30 (0.76–2.22)	1.39 (0.82–2.38)	2.02 (1.20–3.38)	0.02
Model 4 <sup>d</sup> , RR (95% CI)	1.00	1.50 (0.88–2.55)	1.31 (0.76–2.26)	1.50 (0.87–2.59)	2.18 (1.28–3.73)	0.001

<sup>a</sup>RR was adjusted for age.<sup>b</sup>RR was adjusted for age, years of smoking, and BMI.<sup>c</sup>RR was adjusted for age, years of smoking, BMI, and serum levels of sRAGE (for CML-AGE) or CML-AGE (for sRAGE).<sup>d</sup>RR was adjusted for log-transformed glucose levels based on model 3.

assayed at the earlier time point, as well as in the lag analyses. For example, when we excluded men with less than 10 years of follow-up, the RR (95% CI) for the fifth quintile of sRAGE compared with first quintile was 0.35 (0.17–0.73; *P*<sub>trend</sub> < 0.0001). Notably, we found that high levels of CML-AGE were associated with statistically significantly reduced risk of pancreatic cancer [RR (95% CI): 0.47 (0.24–0.91), fifth compared with first quintile, *P*<sub>trend</sub> = 0.01] even after adjustment for sRAGE. The exclusion of participants with self-reported diabetes had no influence on the results. When we limited analyses in the study subjects with less than 3 freeze-thaw

cycles of sera (147 cases and 454 subcohort participants), the RR for pancreatic cancer related to CML-AGE and sRAGE was 0.69 (95% CI: 0.29–1.68, *P*<sub>trend</sub> = 0.52) and 0.39 (95% CI: 0.18–0.86, *P*<sub>trend</sub> < 0.001), respectively, after adjustment for the freeze-thaw cycles, fifth compared with first quintile.

## Discussion

In this prospective study, consistent with our hypothesis, serum levels of sRAGE had an inverse association with the risk of pancreatic cancer. This association was independent of

**Table 4.** Joint effects of CML-AGE and sRAGE and risk of pancreatic cancer

CML-AGE	sRAGE	Case ( <i>n</i> )	Subcohort ( <i>n</i> )	RR (95% CI) <sup>a</sup>
<Median <sup>b</sup>	≥Median	26	49	1.00
≥Median	≥Median	60	143	1.41 (0.82–2.45)
<Median	<Median	144	116	3.00 (1.79–5.03)
≥Median	<Median	53	99	2.07 (1.17–3.67)

NOTE: *P* value for interaction was 0.048.<sup>a</sup>RR was adjusted for age, BMI, and years of smoking.<sup>b</sup>Median value of CML-AGE or sRAGE for the subcohort participants was used as the cutoff point.

CML-AGE and glucose. In contrast to our expectation, higher levels of CML-AGE were not associated with a higher risk of pancreatic cancer. However, the participants with a high CML-AGE/sRAGE ratio had a significantly higher risk of pancreatic cancer. The association between CML-AGE and pancreatic cancer risk differed by levels of sRAGE.

Several recent investigations indicate that RAGE plays a potentially important role in carcinogenesis (17, 19–22). The engagement of full-length RAGE by CML-AGE triggers rapid generation of intracellular reactive oxygen species and activates an array of key cell-signaling pathways that have been implicated in oncogenesis. These cascades set the stage for sustained activation of innate immune response and proinflammatory reactions (32). A role of AGEs/RAGE in pancreatic carcinogenesis has been suggested. One study conceived that chronic exposure to AGEs in prolonged hyperglycemic states could drive tumor growth. This study showed that AGEs–RAGE interaction stimulates the growth of human pancreatic cancer cells MIA PaCa-2 through the autocrine induction of platelet-derived growth factor-B (33). High RAGE expression levels have been correlated with the metastatic potential of pancreatic carcinoma cell lines (34). Furthermore, the role of AGEs/RAGE in pancreatic carcinogenesis is indirectly supported by a few epidemiologic observations. The use of metformin has been shown to reduce pancreatic cancer risk among diabetics (35). Metformin is a potent inhibitor of glycation (36) and has been shown to reduce bovine endothelial cell protein expression of RAGE (37). In 2 prospective studies, tooth loss and periodontal diseases have been positively associated with pancreatic cancer risk (38, 39). RAGE may play a role in the progression of periodontal diseases exacerbated by smoking (40). Taken together, there are several lines of evidence that support the potential involvement of the AGEs–RAGE axis in pancreatic cancer development.

sRAGE binds RAGE ligands, such as CML-AGE, and blocks the engagement of RAGE and ligands. Therefore, sRAGE may be an endogenous protective factor against the effects mediated by RAGE (18). With few exceptions (41, 42), many studies have shown an inverse association between circulating levels of sRAGE and type 2 diabetes (18). Lower levels of serum sRAGE have been linked to poor glycemic control among patients with type 2 diabetes (43). Although the functional role of sRAGE in the circulation remains to be elucidated, we speculated that the chronic inflammatory environment fostered by AGEs–RAGE interaction may be conducive to tumor growth, whereas sRAGE may be an inhibitory influence.

The lack of a positive association between CML-AGE and risk of pancreatic cancer may be attributable to the detoxification or neutralization of CML-AGE by sRAGE or other receptors of CML-AGE (44). The trend of an inverse association between CML-AGE and pancreatic cancer risk may be mediated through sRAGE as the association was attenuated and became nonsignificant after adjustment for sRAGE. We observed a borderline, significant, sub-multiplicative joint effect between CML-AGE and sRAGE on pancreatic cancer. This result suggested that lower levels of sRAGE rather than higher levels of CML-AGE was associated with pancreatic

cancer. Low sRAGE was strongly associated with an increased risk of pancreatic cancer when CML-AGE was low. High CML-AGE was not associated with an increased risk. Nevertheless, we observed a positive association between the CML-AGE/sRAGE ratio and pancreatic cancer risk. Although speculative, it is possible that unbound CML-AGE is involved in pancreatic carcinogenesis. These aspects may be clarified with additional epidemiologic studies, as well as basic research.

Our study had several limitations. First, as smokers have a higher burden of oxidative stress, our findings may not be generalizable to other populations that include nonsmokers or women and, therefore, need to be confirmed in other study populations. Nevertheless, the positive correlation between circulating levels of CML-AGE and sRAGE observed in this study has also been reported in other study populations (45, 46). Second, we only had a single measurement of these biomarkers prior to diagnosis of pancreatic cancer. Repeated measurements within the same individual over time would provide a better picture for the risk assessment. Third, although CML-AGE is a prominent type of AGEs compounds, it is unknown how well it reflects the total burden of AGEs exposure that was not quantified by this study and may contribute to cancer development (47). Fourth, it is possible that the risk estimates were influenced by measurement imprecision due to the freeze-thaw cycle of serum or ELISA error; however, the risk estimates after exclusion of subjects whose serum have more than 2 freeze-thaw cycles were similar to those in all study subjects. Finally, the interpretations of the study findings are limited by the present incomplete understanding of the dynamic interaction of sRAGE with ligands of RAGE in cancer development. It is possible that sRAGE is associated with other ligands of RAGE or interacts with unmeasured molecules in a different pathway.

In summary, this prospective study supports the hypothesis that sRAGE is inversely associated with pancreatic cancer. Future studies should investigate the correlation between markers of insulin resistance, inflammation, and oxidative stress and sRAGE and their interactions in relation to pancreatic cancer. Understanding the genetic and environmental factors (such as dietary intake and cigarette smoking) that regulate the RAGE–ligand axis may provide an insight into new etiologic factors.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

#### Acknowledgements

We thank the participants of the ATBC Study. We also thank Kirk Snyder, Dominick Parisi, and Jason Ashby at the Information Management Services, Inc. for data management support; Karon Drew and the staff at NCI Frederick Central Repository Services for sample retrieval; Dr. William C. Kopp, Timothy Sheehy, and the staff at SAIC-Frederick for collecting pilot study samples and sample aliquot; Dr. Todd M. Gibson at Nutritional Epidemiology Branch for testing the batch effect of insulin and glucose concentrations; and Tawanda Roy at the Nutritional Epidemiology Branch for administrative assistance. We thank Dr. Hashem B. El-Serag at the Baylor College of Medicine for his support in manuscript writing.

## Grant Support

This work was supported by the intramural research program of the Division of Cancer Epidemiology and Genetics of the National Cancer Institute at the NIH. The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study was supported by U.S. Public Health Service contracts N01-CN-45165, N01-RC-45035, N01-RC-37004, and HSN26120100006C from the National Cancer Insti-

tute, U.S. Department of Health and Human Services, and by funding from the Intramural Research Program of the National Cancer Institute.

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Received July 19, 2010; revised February 26, 2011; accepted March 18, 2011; published online May 3, 2011.

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