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Original Article

DNA methylation of oxidative stress genes and cancer risk in the Normative Aging Study

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Abstract: Oxidative stress (OS) is a primary mechanism of carcinogenesis, and methylation of genes related to it may play a role in cancer development. In this study, we examined the prospective association between blood DNA methylation of four oxidative stress genes and cancer incidence. Our study population included a total of 582 participants in the Normative Aging Study (NAS) who had blood drawn during 1-4 visits from 1999-2012 (mean follow up 9.0 years). Promoter DNA methylation of *CRAT*, *iNOS*, *OGG1* and *GCR* in blood leukocytes was measured using pyrosequencing. We used Cox regression models to examine prospective associations between cancer incidence and both methylation at the baseline visit and methylation rate of changes over time. Baseline *OGG1* methylation was associated with higher risk of all-cancer (HR: 1.43, 95% CI: 1.15-1.78) and prostate cancer (HR: 1.52, 95% CI: 1.03-2.25) incidence. Compared with participants remaining cancer-free, those who eventually developed cancer had significantly accelerated *CRAT* methylation ($p = 0.04$) and decelerated *iNOS* methylation ($p<0.01$) over time prior to cancer diagnosis. Accelerated *CRAT* methylation was associated with higher all-cancer incidence (HR: 3.88, 95% CI: 1.06-14.30), whereas accelerated *iNOS* methylation was associated with lower all-cancer incidence (HR: 0.08, 95% CI 0.02-0.38). Our results suggest that methylation and its dynamic change over time in OS-related genes, including *OGG1*, *CRAT* and *iNOS,* may play an important role in carcinogenesis. These results can potentially facilitate the development of early detection biomarkers and new treatments for a variety of cancers.

Keywords: DNA methylation, oxidative stress, cancer incidence

Introduction

Oxidative stress (OS) refers to an imbalance between the production of free radicals and reactive metabolites (such as oxidants or reactive oxygen species) and their elimination by protective mechanisms [1]. OS, which is produced under sustained environmental stress, can in turn induce several carcinogenetic mechanisms, such as somatic mutations, DNA damage, and genomic instability [2, 3].

DNA methylation plays an important role in the regulation of gene expression, and aberrant methylation is a potential predictor of many forms of cancer [4, 5]. Aberrant methylation of OS genes (e.g., from carcinogenic environmental exposures) can contribute to carcinogenesis by alerting OS-related pathways and further promoting cancer development. For example, altered inducible nitric oxide synthase (*iNOS*) gene expression in cancer tissues can be induced by cancer-causing infectious agents, as well as noninfectious agents such as asbestos, and may play a role in inflammation-induced cancer promotion [6]. Reduced DNA repair by 8-oxoguanine DNA glycosylase (*OGG1*) has been suggested as one possible mechanism by which folate deficiency increases cancer risk. [7]. Overexpression of several OS biomarkers

was identified in tumor samples of renal cell carcinoma patients, suggesting that an examination of these genes may hold promise for the early detection of cancer [8].

However, previous studies are largely retrospective in nature, relying upon methylation measured after cancer diagnosis, in which case aberrant DNA methylation could be a consequence of cancer development and/or treatment, instead of a cause or mediator of environmental carcinogens. In general, previous studies are also limited to examining DNA methylation at one time point only, which are less informative regarding the dynamic relationship between DNA methylation and cancer development. Therefore, our objective is to examine prospective associations between risk of developing cancer and blood leukocyte DNA methylation of a panel of genes related to OS: carnitine O-acetyltransferase (*CRAT*), inducible nitric oxide synthase (*iNOS*), 8-oxoguanine DNA glycosylase (*OGG1*) and glucocorticoid receptor (*GCR*) in a longitudinal cohort study.

Materials and methods

Study population

The Normative Aging Study (NAS) was established by the US Department of Veteran Affairs in 1963 with an initial cohort of 2280 healthy men [9]. Initial eligibility criteria at enrollment included veteran status, residence in the Boston area, age 21-80, and no history of hypertension, heart disease, cancer, diabetes, or other chronic health conditions. Participants were recalled for clinical examinations every 3-5 years. Starting in 1999, participants were asked at each visit to donate a 7-ml blood sample for genetic and epigenetic analysis. From 1999 through 2012, 802 of 829 (96.7%) NAS participants regularly attending study follow-up visits agreed to this donation. Of these, 582 (72.6%) participants were free of cancer at baseline (defined as the first visit that included a blood sample for DNA analysis) and included in our study. This study was approved by the Institutional Review Boards of all participating institutions, and written consent forms obtained from all participants.

NAS visits also collected data on subject characteristics through anthropometric measurements, medical history questionnaires, and standardized medical exams. For analytic purposes we dichotomized race as white or non-white, and collapsed educational status into three groups (<13 years, 13-16 years, >16 years). For cigarette smoking, we considered self-reported status (never *vs.* current *vs.* former), and cumulative pack-years of smoking. Alcohol intake was assessed by self-reported number of servings per day, and dichotomized into drinking 0-1 drinks *vs.* two or more drinks per day on average.

Cancer diagnoses of participants were obtained from questionnaires and confirmed via medical records and histological reports. Among the 582 participants free of cancer at baseline, 137 (23.5%) developed cancer during a mean 9.0 years of follow up including: 47 prostate cancers, 43 skin cancers, and 47 other cancers.

DNA methylation measurement

For the measurement of DNA methylation, DNA was extracted from the buffy coat of 7 ml of stored, frozen whole blood through the use of QiAmp DNA blood kits (QIAGEN, Valencia, CA, USA). The extracted DNA (500 ng; concentration: 50 ng/ml) was treated with the EZ DNA Methylation-Gold Kit (Zymo Research, Orange, CA, USA) according to the manufacturer's protocol. Final elution was done with 30 ml of M-Elution Buffer (Zymo Research). DNA methylation was quantified with bisulfite treatment and simultaneous polymerase chain reaction (PCR) and by pyrosequencing, using previously described primers and conditions [10, 11]. A 50-μl PCR was done in 25 μl of GoTaq Green Master mix (Promega, Madison, WI, USA), 1 pmol biotinylated forward primer, 1 pmol reverse primer, 50 ng bisulfite-treated genomic DNA, and water. The degree of methylation was expressed as the proportion of cytosines that were 5-methylated (%5mC). Non-CpG cytosine residues were used as built-in controls to verify bisulfite conversion. Methylation measurements were standardized by processing batch number to have a mean value of 0 and a standard deviation of 1. Candidate genes were identified through a literature review of genes involved in oxidative stress pathways. The assays for methylated DNA were designed to cover the greatest possible number of CpG sites within the promoter region, taking into account the necessary length of the PCR ampli-

con, length of the target sequence, and primers that avoided CpGs. We measured DNA methylation levels at multiple CpG sites (one CpG site for *GCR*, two CpG sites each for *iNOS* and *CRAT*, and four CpG sites for *OGG1*), and calculated mean methylation values for each gene. Exact sites within promoter regions have been previously described [12-14].

Statistical analysis

We assessed variation between OS methylation (dichotomized about the median for descriptive analysis only) and participant characteristics using Student's *t*-test and Fisher's exact test for continuous and categorical variables, respectively. We used multiple Cox proportional hazards regression models to estimate associations between baseline OS methylation and risk of developing cancer.

To capture the dynamic change in DNA methylation over the follow-up visits, we also examined the rate of OS methylation change over time, calculated as the slope of repeated measures of methylation. This involved using a simple linear regression model to estimate changes in methylation over time (slope) for all participants with more than one measurement, and subsequently treating this slope value as an independent variable in subsequent Cox regression models. We also compared methylation rate of change for each gene between incident cancer cases and cancer-free participants using Student's *t*-test. In order to properly interpret our rate of change results, we conducted a sensitivity analysis where we ran another series of Cox proportional hazards models on time-dependent methylation, restricted to the subset of the population that had repeated methylation measures during the follow-up period.

Covariates for adjusted models included age at baseline, race, BMI, education, smoking status, pack-years of smoking, alcohol consumption, white blood cell count and proportion of neutrophils. All analyses were performed using SAS (version 9.3, SAS Institute). Two-sided tests were used throughout, and *p*-values less than 0.05 were considered statistically significant.

Results

Characteristics of participants in our study are similar to those that have been reported previously for this cohort [5]. Overall, participants were male, elderly (mean age 72 years) and overwhelmingly Caucasian (96.5%). The mean BMI of participants was 28.3 kg/m^2 (SD = 4.1). Most of the participants (72%) were college educated or more, and the majority (73%) were current or former smokers. **Table 1** shows the results of our descriptive analysis of baseline OS methylation by participant characteristics. Briefly, *iNOS* and *OGG1* methylation varied across education level (p = 0.01 and p = 0.002, respectively), and *OGG1* methylation also varied across white blood cell count (p = 0.03).

Table 2 shows the results of our analysis of baseline OS methylation with risk of developing cancer. High *OGG1* methylation at CpG site 2 was associated with all-cancer (HR: 1.43, 95% CI: 1.15, 1.78) and prostate cancer incidence (HR: 1.52, 95% CI: 1.03, 2.25), but *OGG1* methylation at other sites and on average was not significantly associated with cancer incidence. We likewise found no significant associations between *CRAT*, *iNOS*, and *GCR* methylation at baseline and cancer incidence.

Rate of mean OS gene methylation change were also associated with cancer incidence (**Table 3**). Mean *CRAT* methylation increased in participants who later developed cancer (rate: 0.06 units/year) relative to cancer-free participants (rate: -0.007 units/year; p = 0.04). The rate of mean *CRAT* methylation change was positively associated with all-cancer incidence (HR: 3.88, 95% CI: 1.06, 14.3). Conversely, mean *iNOS* methylation decreased in incident cancer cases (rate: -0.10 units/year) relative to cancer-free participants (rate: 0.02 units/year; p<0.01), and the rate of mean *iNOS* methylation change was inversely associated with all-cancer incidence (HR: 0.08, 95% CI: 0.02, 0.38). These results were similar across all individual CpG sites for *CRAT* and *iNOS* (data available upon request). We found no significant relationships between *OGG1* or *GCR* methylation rates of change and cancer. Sensitivity analysis with time-dependent Cox models showed no significant associations between mean OS gene methylation and cancer incidence in subset of the population with only repeated methylation measures.

Discussion

In the present cohort of elderly men, we assessed for the first time the prospective association between blood leukocyte DNA

Table 1. Subject characteristics by mean OS methylation at baseline

	Total	CRAT			INOS			OGG1			GCR		
	Mean ± SD/n (%)	Low†	High†	p	Low†	High†	p	Low†	High†	p	Low†	High†	p
Age (years)	71.7±6.7	71.7±6.9	71.9±6.7	0.63	72.5±7.2	71.9±6.8	0.44	72.7±7.1	71.8±3.5	0.28	71.9±6.9	71.6±6.6	0.62
Body Mass Index (kg/m^2)	28.3±4.1	28.5±4.1	28.2±4.2	0.45	28.6 (4.6)	28.0 (4.1)	0.18	28.6±4.7	27.9±4.2	0.14	28.3±4.0	28.5±4.4	0.48
Race													
White	556 (95.5)	263 (96.7)	252 (94.7)		176 (96.2)	175 (96.2)		169 (96.6)	161 (97.0)		234 (95.1)	230 (95.4)	
Non-white	26 (4.5)	9 (3.3)	14 (5.3)	0.26	7 (3.8)	7 (3.8)	0.99	6 (3.4)	5 (3.0)	0.83	12 (4.9)	11 (4.6)	0.87
Education (years)													
<13	167 (28.7)	74 (27.2)	82 (30.8)		62 (33.8)	42 (23.1)		68 (38.9)	43 (25.9)		67 (27.2)	75 (31.1)	
13-16	286 (49.1)	140 (51.5)	121 (45.5)		76 (41.5)	104 (57.1)		82 (46.9)	77 (46.4)		125 (50.8)	108 (44.8)	
>16	129 (22.2)	58 (21.3)	63 (23.7)	0.38	45 (24.6)	36 (19.8)	0.01*	25 (14.3)	46 (27.7)	0.002*	54 (22.0)	58 (24.1)	0.41
Smoking status													
Never	160 (27.5)	86 (31.6)	65 (24.4)		43 (23.5)	45 (24.7)		43 (24.6)	40 (24.1)		72 (29.3)	64 (26.6)	
Current	27 (4.6)	12 (4.4)	12 (4.5)		7 (3.8)	8 (4.4)		9 (5.1)	8 (4.8)		13 (5.3)	12 (5.0)	
Former	395 (67.9)	174 (64.0)	189 (71.1)	0.18	133 (72.7)	129 (70.9)	0.92	123 (70.3)	118 (71.1)	0.98	161 (65.5)	165 (68.5)	0.78
Pack-years of smoking	20.7±24.1	20.0±24.6	22.0±23.6	0.36	21.3±24.3	22.2±18.6	0.74	20.0±25.6	21.1±23.4	0.72	20.3±23.9	22.2±25.6	0.39
Mean alcohol consumption													
0-1 drinks/day	480 (82.5)	222 (81.6)	220 (82.7)		150 (82.0)	148 (81.3)		143 (81.7)	128 (77.1)		204 (82.9)	199 (82.6)	
2+ drinks/day	102 (17.5)	50 (18.4)	46 (17.3)	0.74	33 (18.0)	34 (18.7)	0.87	32 (18.3)	38 (22.9)	0.29	42 (17.1)	42 (17.4)	0.92
White blood cell count	6.4±2.2	6.4±1.6	6.3±1.6	0.50	6.6±3.1	6.3±1.6	0.29	6.8±3.1	6.2±1.6	0.03*	6.4±1.5	6.4±1.7	0.96
Neutrophils proportion	62.0±8.3	61.8±7.9	62.4±8.0	0.46	61.4±8.5	62.6±7.8	0.18	62.4±9.0	62.1±8.7	0.79	62.8±7.8	61.6±8.0	0.13

† = Methylation subgroups were divided by median methylation for each gene at the baseline. * = Statistically significant at p<0.05; *P*-values shown for Student's t-test and Fisher's exact test for continuous and categorical characteristics, respectively.

Table 2. Associations between baseline OS methylation and cancer incidence

		All cancer			Prostate cancer		
	Cancer-free	n	HR (95% CI)	p	n	HR (95% CI)	p
CRAT							
Site 1	403	119	0.95 (0.79-1.15)	0.61	41	1.22 (0.93-1.59)	0.15
Site 2	403	119	0.83 (0.68-1.12)	0.07	41	0.87 (0.62-1.22)	0.42
Mean	403	119	0.87 (0.71-1.05)	0.15	41	0.96 (0.69-1.32)	0.81
INOS							
Site 1	285	70	1.10 (0.82-1.45)	0.54	26	1.33 (0.86-2.07)	0.2
Site 2	285	70	1.21 (0.91-1.58)	0.18	26	1.56 (0.98-2.47)	0.06
Mean	285	70	1.19 (0.90-1.57)	0.23	26	1.52 (0.97-2.40)	0.07
OGG1							
Site 1	261	68	0.8 7 (0.67-1.13)	0.29	23	1.02 (0.67-1.54)	0.94
Site 2	261	68	1.43 (1.15-1.78)	0.001*	23	1.52 (1.03-2.25)	0.03*
Site 3	261	68	0.91 (0.70-1.17)	0.46	23	0.80 (0.49-1.31)	0.37
Site 4	261	68	1.01 (0.80-1.28)	0.9	23	1.02 (0.68-1.52)	0.91
Mean	261	68	1.05 (0.84-1.34)	0.64	23	1.14 (0.77-1.69)	0.52
GCR							
Site 1	362	110	0.90 (0.74-1.10)	0.32	35	0.91 (0.64 -1.31)	0.62

* = Statistically significant at p<0.05.

methylation of OS genes and cancer risk. In our study, only baseline *OGG1* methylation at CpG site 2 was associated with risk of both all-cancer and prostate cancer incidence. We also found that participants who subsequently developed cancers experienced increased *CRAT* methylation over time and decreased *iNOS* methylation while cancer-free participants experienced relatively stable methylation levels of both genes. Also rates of change of *CRAT* and *iNOS* methylation were significantly associated with all-cancer incidence. The dynamics of these genes' methylation suggests that the ongoing epigenetic dysregulation of OS genes could be an important contributor to the development of cancer.

Our finding of increased all-cancer and prostate cancer incidence among participants with higher baseline *OGG1* methylation in a single promoter CpG site supports several previous studies of *OGG1*. As a gene with DNA-repair function, *OGG1* encodes 8-oxoguanine DNA glycosylase, which is involved in the excision of 8-oxoguanine, [15] itself a common mutagenic DNA lesion that is formed by intracellular oxidation and/or exposure to external carcinogens [16-18]. Lower 8-oxoguanine DNA glycosylase activity, and consequent reduced ability to repair OS-related DNA damage, has been associated with cancers of the lung, kidney, head and neck [15, 19, 20]. Reduced OGG1 activity has also been associated with UV exposure *in vitro*. [21]. To our knowledge, our study is the first to link cancer-related changes in circulating 8-oxoguanine DNA glycosylase with epigenetic alterations. However, only *OGG1* methylation measured at a single site was significantly associated with cancer risk in our study. Two prior studies showed that methylation of the promoter regions of genes is frequently site-specific and that certain methylating CpG sites could have a stronger impact on transcriptional suppression than others [14, 22]. These findings suggest that there may be biologically and/or clinically relevant heterogeneity of effects in the methylation of different CpG sites within *OGG1*. Alternatively, the weakness of these findings may be a reflection of our cohort data. Older men were found to have reduced *OGG1* expression in one study using blood cells, [23] and another suggested that blood leukocytes have lower *OGG1* expression in healthy subjects [24]. Future research to examine site #2 of *OGG1* in greater detail and utilizing different cell types (as well as cell-free DNA), and to confirm its relationship with cancer development, is warranted. This finding may be driven in part by the high proportion of skin cancers in our study; studying this position of *OGG1* in the context of UV exposure and melanoma may be particularly informative. Moreover, the positive association with prostate cancer, itself consistent with a prior finding of elevated markers of oxidative DNA damage in the blood of prostate cancer patients, [25] suggest that *OGG1* may be useful as a potential biomarker of multiple different types of cancer.

In addition, we also observed increased *CRAT* gene methylation in incident cancer cases com-

Table 3. OS methylation rate of change and cancer incidence

	Cancer-free	All-cancer	p
CRAT			
N	291	56	
Mean (units/year)	-0.007	0.06	0.035*
HR (95% CI)	REF	3.88 (1.06-14.3)	0.041*
INOS			
N	222	39	
Mean (units/year)	0.017	-0.10	0.0037*
HR(95% CI)	REF	0.08 (0.02-0.38)	0.001*
OGG1			
N	192	38	
Mean (units/year)	-0.03	-0.05	0.77
HR(95% CI)	REF	0.64 (0.11-3.75)	0.62
GCR			
N	269	55	
Mean (units/year)	-0.02	0.01	0.52
HR (95% CI)	REF	1.51 (0.47-4.87)	0.49

* = Statistically significant at p<0.05.

pared to those who remained cancer-free. *CRAT* produces a mitochondrial matrix enzyme that catalyzes the inter-conversion of acetyl-CoA and acetylcarnitine, playing an important role in numerous metabolic processes. Studies have shown that *CRAT* not only buffers the mitochondrial acetyl-CoA pool but also regenerates free CoA, both of which influence the activities of several oxidative enzymes [26]. Insufficient *CRAT* activity could exacerbate metabolic derangements and further increase oxidative stress [27]. A prior NAS analysis by Madrigano, et al. showed that aging, a known risk factor for cancer, was significantly associated with increased *CRAT* methylation [28]. However, the fact that the rate of *CRAT* methylation significantly differed between participants developing cancer and cancer-free participants means that the association between *CRAT* and cancer cannot be explained entirely by the aging process. Animal models have shown an inverse relationship between *CRAT* activity and BMI, suggesting a possible link to nutritional and/or other lifestyle factors that were beyond the scope of this analysis [27]. Other potential causes of accelerated *CRAT* methylation, such as nutrition or environmental exposures, should be further explored to inform the involvement of oxidative stress processes in cancer development over time as well as to develop a potentially useful biomarker of poor nutrition or other carcinogenic exposure.

Nitric oxide (NO) is another free radical implicated in carcinogenesis. NO has deleterious effects on many cell components including DNA [29] and the majority of NO in the human body is synthesized by *iNOS*, the inducible form of nitric oxide synthase [30]. The expression of *iNOS* could be stimulated by many factors including immunological or inflammatory stimuli like γ-interferon, TNF-α, IL1 and so on. [31], [32] *iNOS* expression has been found to be increased in various types of cancer including lung, [33] prostate, [34-36] and colorectal cancers [37]. A comparison of human and murine models has suggested that *iNOS* expression is particularly susceptible to the effects of changes in methylation [38]. The small hazard ratio in our study for the rate of *iNOS* methylation change and cancer development supports this notion. Increased expression of *iNOS* could also promote tumor growth via the stimulation of tumor angiogenesis, which is a requirement for the growth of solid tumors [39-41]. In addition, NO's role as a cancer promoter suggests that this decreasing *iNOS* methylation could be the result of carcinogenic exposures [42]. *iNOS* and NO pathways have been suggested as one possible route through which nutritional factors influence the risk of chronic inflammation-related diseases, including cancer [43]. This could also explain the association between *iNOS* and education seen in our descriptive analysis, with education serving as a proxy for poor nutrition and *iNOS* mediating those effects on cancer risk. Further studies of *iNOS* methylation in the context of poor nutrition and other, more direct, measures of socioeconomic status will help to confirm its involvement in this pathway, and may allow for the development of new health interventions to partially compensate for some of the health effects of poor nutrition. Finally, *iNOS*' susceptibility to methylation effects coupled with its involvement in a number of different tumorigenic processes makes it a strong candidate for chemoprevention and/or chemotherapy [44]. If methylation of *iNOS* could be induced, it could potentially impact cancers of a number of different organ sites.

Our rate-of-change analyses identified relatively rapid increases in *CRAT* methylation, and relatively rapid decreases in *iNOS* methylation, for both the average methylation value and all position-specific values, but only among the population that at some point developed cancer during the follow-up period. To our knowledge, no other study has identified trends over time in gene-specific blood leukocyte DNA methylation during the process of cancer development. If verified in other, more diverse populations, these two genes could potentially provide a valuable measurement of oxidative stress in the body, and serve as a useful biomarker of cancer prediction or risk stratification for screening (particularly since methylation of these genes in cancer-free participants was relatively stable). Being able to reactivate DNA repair mechanisms specific to OS may also provide valuable new avenues for the development of new treatments for cancer, at least one of which (based on NO pathways) has already showed preliminary success *in vitro*. [45]. The fact that the rate of change for both *CRAT* and *iNOS* methylation, rather than time-dependent or baseline methylation measures, suggests that these genes may not be mechanistically involved in the development of cancer. Rather, these epigenetic changes over time may be reflective of other biological processes, possibly related to oxidative stress, that are occurring as a precursor to or part of carcinogenesis. The role of *iNOS* and NO in cancer development is complex, and dependent on a variety of different factors, some of them time-dependent [46]. Additional longitudinal studies of OS gene methylation are necessary to establish these genes' value as a biomarker of cancer and potential utility as a chemopreventative agent, and to further clarify the relationship between OS gene methylation rate of change, and cancer development.

The longitudinal nature of this study enabled us to explore the temporal associations between OS methylation and cancer risk while avoiding the biases often encountered in cross-sectional or retrospective studies. However, our study was subject to several limitations. We measured OS methylation from peripheral blood leukocytes. While OS methylation in leukocytes could show the cumulative effect of general environmental and lifestyle factors, it could be different from tissue- or tumor-specific OS methylation profiles. However, all the genes we selected were known to be expressed and functional in leukocytes and participate in critical cellular functions, measurement in leukocytes may be appropriate biological markers. In addition, our study participants were all male and majority Caucasian, thus further studies in female and non-Caucasian populations are warranted to confirm our findings. Finally, although our study has a large quantity of data and multiple follow-up measurements, the sample size in our study limited our ability to study specific cancer types other than prostate cancer. Thus, caution should be exercised when interpreting our results as different types of cancer may have different causes and mechanisms. Larger studies with multiple cancer types are necessary to confirm the value of OS methylation as universal cancer biomarkers.

In conclusion, our results suggest that methylation in key OS genes may play an important role in carcinogenesis. *OGG1* methylation at baseline and rates of change of mean methylation for both *CRAT* and *iNOS* genes in the years prior to cancer diagnosis are associated with cancer incidence. Furthermore, ongoing methylation change for *CRAT* and *iNOS* seems to behave qualitatively differently between participants developing cancer and those who remain cancer free, suggesting ongoing biological processes measurable in peripheral blood cells that are linked to cancer development and pointing to a potentially viable biomarker of multiple different types of cancer. Further study of the processes associated with *CRAT* and *iNOS* could potentially inform our understanding of carcinogenesis and lead to new interventions to prevent cancer.

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Disclosure of conflict of interest

None.

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