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Antibody Responses to *Fusobacterium nucleatum* Proteins in Prediagnostic Blood Samples are not Associated with Risk of Developing Colorectal Cancer

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Abstract

Background: There is a lack of prospective data on the potential association of *Fusobacterium nucleatum* (*F. nucleatum*) and colorectal cancer risk. In this study, we assessed whether antibody responses to *F. nucleatum* are associated with colorectal cancer risk in prediagnostic serum samples in the European Prospective Investigation into Nutrition and Cancer (EPIC) cohort.

Methods: We applied a multiplex serology assay to simultaneously measure antibody responses to 11 *F. nucleatum* antigens in prediagnostic serum samples from 485 colorectal cancer cases and 485 matched controls. Conditional logistic regression models were used to estimate odds ratios (ORs) and 95% confidence intervals (CI).

Results: We observed neither a statistically significant colorectal cancer risk association for antibodies to individual *F. nucleatum* proteins nor for combined positivity to any of the 11 proteins (OR, 0.81; 95% CI, 0.62–1.06).

Conclusions: Antibody responses to *F. nucleatum* proteins in prediagnostic serum samples from a subset of colorectal cancer cases and matched controls within the EPIC study were not associated with colorectal cancer risk.

Impact: Our findings in prospectively ascertained serum samples contradict the existing literature on the association of *F. nucleatum* with colorectal cancer risk. Future prospective studies, specifically detecting *F. nucleatum* in stool or tissue biopsies, are needed to complement our findings.

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Introduction

Several studies reported an overabundance of *Fusobacterium nucleatum* (*F. nucleatum*) in stool and tumor tissue of patients with colorectal cancer (1, 2). Although mechanistic studies suggest an etiologic role of the bacterium in colorectal carcinogenesis, it remains possible that it is simply an opportunistic passenger within developing tumors (1). To our knowledge, *F. nucleatum* has never been studied in relation to colorectal cancer risk using prediagnostic biological samples.

Multiplex serology provides an easy-to-apply tool to assess potential infection-associated cancers in large prospective epidemiologic studies that provide sufficient statistical power (3).

In this study, we assessed whether antibody responses to *F. nucleatum* proteins are associated with colorectal cancer risk in prediagnostic serum samples from 485 colorectal cancer cases and 485 controls in the European Prospective Investigation into Nutrition and Cancer (EPIC) cohort.

Materials and Methods

Study population

The colorectal cancer cases and controls in this nested case–control study are participants within the EPIC study (4). The study was approved by the International Agency for Research on Cancer Ethics Committee (Lyon, France) and the ethics committees of all local EPIC centers, and performed in accordance with the Helsinki Declaration.

This study included prediagnostic serum samples from 485 colorectal cancer cases with a median time between blood draw and diagnosis of 3.4 years (range 0.4–8.5 years; primary tumors coded C18-C20 according to the 10th revision of the International Statistical Classification of Diseases, Injury and Causes of Death). Controls were selected by incidence density sampling from all cohort members alive and cancer-free at the time of matching to cases (1:1) as described previously, including age and sex (5).

F. nucleatum multiplex serology

Serum samples were analyzed for antibodies against *F. nucleatum* proteins in a 1:1,000 dilution using the previously described multiplex serology method (3). We selected 11 *F. nucleatum* (strain ATCC25586) proteins as antigens (Table 1). Antigen-specific cut-off values were defined arbitrarily by visual inspection of percentile plots at the approximate inflection point of the curve. Antibody responses to *F. nucleatum* proteins were combined to overall *F. nucleatum* sero-positivity by assessing positivity to at least one, and to increase specificity, to at least two or three proteins.

Statistical analysis

Conditional logistic regression models were applied to compute odds ratios (ORs) and 95% confidence intervals (CI) for the association of antibody responses to *F. nucleatum* proteins with colorectal cancer risk, overall and by time between blood draw and diagnosis. The following variables were considered as potential confounders and included in the model for adjustment: body mass index (BMI), smoking status, alcohol consumption, and highest education attained at baseline.

All statistical analyses were performed with SAS version 9.4 (SAS Institute). A P value below 0.05 was considered statistically significant.

Availability of data and materials

For information on how to submit an application for gaining access to EPIC data and/or biospecimens, please follow the instructions at http://epic.iarc.fr/access/index.php.

Results

Sero-prevalence to individual *F. nucleatum* proteins ranged between 3% and 10% among controls (Table 2). Fifty-three percent of controls were positive to any of the proteins; this number was reduced for positivity to at least two (21%) or three proteins (9%). We did not identify a higher sero-prevalence among colorectal cancer cases, neither to individual proteins (range 2%–11%), nor positivity to any (47%) or several proteins ($\geq$2 proteins: 17%; $\geq$3 proteins: 9%). Thus, there was no significant positive association of antibodies to *F. nucleatum* proteins with colorectal cancer risk. Sero-positivity to FN0131 was inversely related with colorectal cancer risk (OR, 0.61; 95% CI, 0.38–0.98; $P = 0.042$; Table 2). However, this result is not significant after Bonferroni multiple-testing correction. Results did not vary for individuals diagnosed within or after more than 2 years of diagnosis (Table 2).

Discussion

In this multi-center prospective study, we found that antibody responses to *F. nucleatum* proteins were not associated with colorectal cancer risk.

Our findings are discordant to the published literature that suggests a role for *F. nucleatum* in colorectal cancer development. Our study design varies from previous literature in two important ways. First, previous human studies mostly measured *F. nucleatum* abundance at the site of interest, that is, in stool or tumor tissue (1), while we apply serology, which is limited by being a systemic measure of past and/or current infection. Thus, it remains possible that the antibody responses resulted from other

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Table 1. *F. nucleatum* (strain ATCC25586) antigens and antigen-specific cut-off values (MFI)

Name	(Predicted) function	Protein accession no.[a]	Selected region (aa)	Antigen-specific cutoff (MFI)
Fn0131	Type Vb secretion system (TpsB)	NP_603038	17–566	86
Fn0253	Outer membrane protein	NP_603160	37–132	30
Fn0264	Adhesin (FadA)	NP_603171	19–129	30
Fn0387	Type Va secretion system	NP_603291	1442–1714	30
Fn1426	Type Va secretion system	NP_604320	25–374	86
Fn1449	Type Va secretion system (Fap2)	NP_604343	2884–3155	33
Fn1526	Type Va secretion system (RadD)	NP_602353	1857–2135	30
Fn1817_1	Type Vb secretion system (TpsA)	NP_602617	205–276	42
Fn1817_2	Type Vb secretion system (TpsA)	NP_602617	839–909	112
Fn1859	Porin (FomA)	NP_602659	21–368	31
Fn1893	Type Va secretion system	NP_602692	1079–1351	30

Abbreviations: aa, amino acid; *F. nucleatum, Fusobacterium nucleatum*; MFI, median fluorescence intensity.
[a]NCBI reference sequence.

Table 2. Antibody responses to *F. nucleatum* proteins and colorectal cancer risk, overall and by time between blood draw and diagnosis; the EPIC study, 1992–2003

	All				≤2 years follow-up time				>2–8.5 years follow-up time			
	Positive *n* (%)				Positive *n* (%)				Positive *n* (%)			
	Controls	Cases			Controls	Cases			Controls	Cases		
Antigen	*n* = 485	*n* = 485	OR[a] (95% CI)	*P*	*n* = 130	*n* = 130	OR[a] (95% CI)	*P*	*n* = 355	*n* = 355	OR[a] (95% CI)	*P*
Fn0131	46 (9)	31 (6)	**0.61 (0.38–0.98)**	**0.042**	13 (10)	10 (8)	0.66 (0.26–1.68)	0.387	33 (9)	21 (6)	0.61 (0.34–1.09)	0.096
Fn0253	15 (3)	10 (2)	0.64 (0.28–1.45)	0.283	3 (2)	2 (2)	0.73 (0.11–4.74)	0.741	12 (3)	8 (2)	0.67 (0.27–1.68)	0.393
Fn0264	24 (5)	32 (7)	1.33 (0.75–2.34)	0.331	5 (4)	7 (5)	1.08 (0.26–4.51)	0.921	19 (5)	25 (7)	1.37 (0.73–2.56)	0.334
Fn0387	38 (8)	42 (9)	1.05 (0.66–1.67)	0.847	10 (8)	17 (13)	1.39 (0.56–3.45)	0.480	28 (8)	25 (7)	0.89 (0.50–1.58)	0.688
Fn1426	49 (10)	53 (11)	1.06 (0.69–1.63)	0.777	15 (12)	11 (8)	0.79 (0.32–1.94)	0.601	34 (10)	42 (12)	1.33 (0.80–2.20)	0.273
Fn1449	47 (10)	41 (8)	0.80 (0.51–1.27)	0.347	12 (9)	16 (12)	1.26 (0.51–3.15)	0.617	35 (10)	25 (7)	0.68 (0.39–1.18)	0.165
Fn1526	20 (4)	15 (3)	0.74 (0.37–1.49)	0.401	4 (3)	4 (3)	0.87 (0.18–4.28)	0.868	16 (5)	11 (3)	0.71 (0.32–1.61)	0.414
Fn1817_1	49 (10)	40 (8)	0.82 (0.52–1.29)	0.384	17 (13)	11 (8)	0.63 (0.27–1.51)	0.304	32 (9)	29 (8)	0.97 (0.55–1.70)	0.907
Fn1817_2	48 (10)	46 (9)	1.00 (0.66–1.52)	0.994	14 (11)	12 (9)	0.87 (0.36–2.14)	0.768	34 (10)	34 (10)	1.04 (0.64–1.69)	0.888
Fn1859	46 (9)	34 (7)	0.69 (0.43–1.11)	0.128	8 (6)	7 (5)	0.80 (0.25–2.61)	0.715	38 (11)	27 (8)	0.67 (0.39–1.14)	0.137
Fn1893	47 (10)	45 (9)	0.93 (0.60–1.45)	0.757	11 (8)	15 (12)	1.14 (0.44–2.96)	0.792	36 (10)	30 (8)	0.82 (0.49–1.39)	0.468
Any protein	255 (53)	230 (47)	0.81 (0.62–1.06)	0.130	71 (55)	57 (44)	0.61 (0.35–1.08)	0.090	184 (52)	173 (49)	0.91 (0.66–1.24)	0.546
≥2 Proteins	103 (21)	84 (17)	0.73 (0.53–1.02)	0.066	27 (21)	26 (20)	0.85 (0.44–1.64)	0.618	76 (21)	58 (16)	0.72 (0.48–1.08)	0.112
≥3 Proteins	44 (9)	46 (9)	0.95 (0.61–1.47)	0.818	10 (8)	17 (13)	1.23 (0.49–3.08)	0.656	34 (10)	29 (8)	0.84 (0.50–1.42)	0.523

NOTE: Control subjects were selected by incidence density sampling from all cohort members alive and free of cancer at the time of matching and matched to cases 1:1 by age at blood collection (±6 months–± 2 years), sex, study center, time of the day at blood collection (±2–4 hours interval), fasting status at blood collection (<3/3–6 hours); among women by menopausal status, and among premenopausal women by phase of menstrual cycle, and hormone therapy use at time of blood collection.
[a]Conditional logistic regression model conditioned on the matching factors with multivariable adjustment for BMI, education, smoking, and alcohol intake at baseline; significant associations ($P < 0.05$) are marked in bold font.

infection sites and/or cross-reactive antibody responses from infection with other closely related bacteria. Second and a major strength of our study is that we employed a prospective design, in contrast to the case–control designs of previous studies to assess whether *F. nucleatum* infection increases colorectal cancer risk. However, the natural history of a potential etiologic role of *F. nucleatum* in colorectal cancer development with respect to timing and molecular pathways is unknown. Longitudinal studies and analyses of molecular colorectal cancer subtypes are needed to address this question in more depth.

In conclusion, antibody responses to *F. nucleatum* proteins in prediagnostic serum samples of the EPIC study were not associated with an increased risk of developing colorectal cancer. Future prospective studies, specifically detecting *F. nucleatum* in stool or tissue biopsies, are needed to help clarify whether *F. nucleatum* plays a role in colorectal tumor development.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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