Fusobacterium nucleatum infection is correlated with tumor metastasis and postoperative survival of colorectal cancer patients in China

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Background: In western patients, the association of *Fusobacterium nucleatum* (*F. nucleatum*) with colorectal carcinoma (CRC) has been established, but the underlying mechanism remains unclear. There is no report about *F. nucleatum* infection status and its potential role in CRC patients in China. This study aimed to evaluate infection status and infection load of *F. nucleatum* and its correlation with clinicopathological features and prognosis of CRC patients in China.

Methods: Fresh tumor and adjacent normal mucosa tissues were obtained at surgery from 152 CRC patients, and then DNA was assayed for infection of *F. nucleatum* by quantitative PCR. Then the association of infection with clinicopathological features and prognosis was evaluated. In addition, MMP9, MMP11, CD45RO and FOXP3 expression levels was assessed by immunohistochemistry to explore the potential role of *F. nucleatum*.

Results: The infection rate of *F. nucleatum* in CRC tissues was significantly higher than that in adjacent normal tissues (77.6% *vs.* 57.2%, P<0.001), and median *F. nucleatum* copy number was also significantly higher in CRC tissues than in adjacent normal tissues (87 *vs.* 37, P<0.001). *F. nucleatum* infection of tumor tissues was related with poorer tumor differentiation (P<0.001), deeper tumor invasion (P<0.001), lymph node metastasis (P=0.01), distant metastasis (P=0.001) and advanced TNM stage (P=0.034). Specimens with higher infection load contained fewer CD45RO⁺ T lymphocytes and more FOXP3⁺ regulatory T lymphocytes than those with lower infection load. And high-copy group had a poorer postoperative survival than the low-copy group (P=0.027). In the subset of CRC patients with *F. nucleatum* infection, tumor stage and *F. nucleatum* infection were independent prognostic factors. Advanced tumor stage and high-load infection were correlated poorer prognosis (P=0.023 and 0.032, respectively).

Conclusions: Infection rate and load of *F. nucleatum* are higher in carcinoma tissues than in adjacent normal tissues from CRC patients in Chian. *F. nucleatum* infection was correlated with tumor metastasis and overall postoperative survival of colorectal cancer patients, so it can help to improve prevention, diagnosis and treatment of CRC in China.

Introduction

Fusobacterium nucleatum (*F. nucleatum*), a gram-negative anaerobic species of the *Bacteroidaceae* family, is one of the most common species of bacteria isolated from dental plaque biofilms. This species has been found to play important roles in pericarditis, appendicitis and several oral diseases (1). For example, *F. nucleatum* can secrete butanoic acids and lipopolysaccharide, which has been proved to inhibit human T-cell responses to mitogens and antigens (2) and to induce apoptotic cell death in peripheral blood mononuclear cells (PBMCs) (3). Moreover, all strains of *F. nucleatum* that have been tested can stimulate epithelial cells to secrete proteolytic enzymes, including Matrix metalloproteinases (MMPs) such as MMP-13, which plays an important part in extracellular matrix (ECM) degradation (4).

Colorectal carcinoma (CRC) is the third leading cause of cancer death in America and the second most prevalent cancer in China (5). The pathogenesis of sporadic CRC remains unclear. Recent studies have shown that the *F. nucleatum* infection rate (prevalence) and infection load (abundance) was higher in CRC than in matched normal tissue from western patients (6,7). In addition, a strain of *F. nucleatum* isolated directly from a CRC sample could invade human colonic epithelial cells (6,7).

Progression and survival of CRC patients is dependent on the complex interaction between tumor and host. The pronounced local inflammatory response intra-tumor and around the tumor is thought to represent the anti-tumor immune response. There has been increasing interest in establishing the cellular composition of immune cell infiltrates and their relationship with survival in CRC patients, and over 140 studies have proved that the number/density of immune cells intratumor and around the tumor is an important hint for prognostics, which may be incorporated into routine clinical assessment. In tumor stroma, inflammatory cell types are mainly T lymphocyte and their subsets, and they are collectively known as the tumor infiltrating lymphocytes (TIL). The density of memory T-lymphocytes (CD45RO positive) can reflect local immune response, and the increasing densities of these long-lived memory T-lymphocytes at both tumor centre and invasive margin was associated with improved survival or reduced metastatic potential (8-13). T regulatory lymphocytes (Treg cells, FOXP3 positive) play a role in the suppression of immune responses to foreign and self-antigenic material, and two studies have reported that high density of Treg cells was a strong prognostic factor associated with poorer survival in primary operable CRC (9,14,15). To date, the prevalence of *F. nucleatum* in CRC patients from Asian, including China, has not been determined, and the biological characteristics of *F. nucleatum* has not been investigated in CRC patients. We therefore assayed tumor and matched normal tissue from 152 CRC patients for the infection rate and infection load of *F. nucleatum*. In order to evaluate the biological impact of this infection, we assayed the abundance of memory and regulatory T lymphocytes in these samples to examine the immune response in primary operable CRC. In addition, we evaluated the expression of the proteolytic enzymes MMP9 and MMP11 to identify whether *F. nucleatum* infection in CRC has a similar role in oral disease.

Methods

Patients and samples

Fresh colorectal adenocarcinoma and corresponding adjacent normal tissues were obtained at surgery from 152 patients who underwent radical surgery at Peking University Cancer Hospital, Beijing, China, between January 2009 and December 2010. All these patients were diagnosed as CRC, and did not receive chemotherapy or radiotherapy before surgery. In addition, for these patients, there is no evidence of concomitant infectious or immunologic diseases and no infection after surgery. These patients included 95 males (62.5%) and 57 females (37.5%), with a median age of 62 years (range, 28–84 years; *Table 1*). Normal tissues (NT) were obtained at least 5.0 cm from the tumor margin, and were confirmed as negative for tumor by histological examination. These patients were followed-up postoperatively, over a period of at least 5 years.

All specimens, obtained immediately after surgical resection, were snap-frozen in liquid nitrogen and stored

Table 1 Demographic and clinical characteristics of CRC patients (N=152)

Clinicopathological features	No. of patients	Percentage (%)
Gender		
Male	95	62.5
Female	57	37.5
Age, years		
<60	70	46.1
≥60	82	53.9
Tumor site		
Right-sided	49	32.2
Left-sided	53	34.9
Rectum	50	32.9
Differentiation		
Well	7	4.6
Moderate	119	78.3
Poor	26	17.1
Depth of invasion		
I	6	4.0
II	21	13.8
III	56	36.8
IV	69	45.4
Lymph node metastasis		
N0	65	42.7
N1	46	30.3
N2	38	25.0
N3	3	2.0
Distant metastasis		
M0	126	82.9
M1	26	17.1
Vascular invasion		
Absent	113	74.3
Present	39	25.7
TNM stage		
I	6	3.9
II	57	37.5
III	64	42.1
IV	25	16.5

at –80 ℃. Sixteen samples used for immunohistochemistry must reach the following requirements: (I) moderately differentiated adenocarcinoma; (II) T3N0M0 stage; (III) *F. nucleatum* infection can be detected in both tumor tissue and NT; (IV) age gap between these 16 patients is less than 5 years (57–60 years).

All clinicopathological features were obtained from patient records. Overall survival (OS) or follow-up time was calculated from the date of surgery to date of death or last follow-up respectively. This study was approved by the institutional review board of Peking University Cancer Hospital (Ethical approval number: 2008110621), and written informed consent was acquired from each patient.

Isolation of genomic DNA

DNA was extracted from tissue samples using Transgene® Easy Pure genomic DNA Kits, according to the manufacturer's instructions. DNA concentration was assayed by Nanodrop 2000 (Thermo scientific). The quality of each DNA sample was examined by PCR, using the human β-actin gene primers 5'-TTAGTTGCGTTACACCCTTTC-3' (forward) and 5'-ACCTTCACCGTTCCAGTTT-3' (reverse), resulting in a 150 bp product.

Cloning a fragment of F. nucleatum nusG gene to prepare loading controls

We randomly evaluated several DNA samples by PCR and chose one sample positive for *F. nucleatum* infection. The PCR product was confirmed by gene sequencing, then amplified and cloned into a plasmid using the TA cloning technique to construct a plasmid containing the 161-bp fragment of the *F. nucleatum* nusG gene. The copy number of the plasmid was calculated according to the formula: copy number=(amount×6.022×10^{23})/(length×1×10^9×650). The plasmid was diluted to 10^8~10^1 copies/μL and used as loading controls.

Quantitative real-time PCR to evaluate F. nucleatum infection

The infection rate and infection load of *F. nucleatum* (ATCC 25586) in samples from CRC patients were analyzed by quantitative real-time PCR, using the primers 5'-CAACCATTACTTTAACTCTACCATGTTCA-3' (forward) and 5'-GTTGACTTTACAGAAGGAGATTATGTAAAAATC-3' (reverse). The resulting 161-bp product matched a sequence

of the nusG gene (GenBank accession AAL94126.1) of *F. nucleatum*, but did not match any sequence in other genes of any other species.

Quantitative real-time PCR was conducted using the ABI 7500 Sequence Detection System (Applied Biosystems). Each 20 µL aliquot of the reaction mixture contained 20 ng DNA, 1.5 µM of each primer, and 10 µL Power SYBR® Green PCR Master Mix (Applied Biosystems Inc., California, USA). The amplification protocol consisted of an initial denaturation at 95 ℃ for 10 min, followed by 40 cycles of denaturation at 95 ℃ for 15 s, annealing at 60 ℃ for 1 min, and extension at 72 ℃ for 15 s. All samples were assayed in triplicate.

Immunohistochemistry

Formalin-fixed, paraffin-embedded CRC and adjacent non-tumor tissue samples were sectioned at 4 µm thickness, deparaffinized, and incubated with 3% hydrogen peroxide for 10 min to inhibit endogenous peroxidases. To assay for MMP9, CD45RO and FOXP3, sections were heated in a microwave oven for antigen retrieval; to assay for MMP11, sections were heated in an autoclave. The samples were incubated with 5% nonfat milk to prevent nonspecific binding and overnight at 4 ℃ with primary antibodies to MMP9 (rabbit monoclonal antibody, 1:200; CST, Boston, MA), MMP11 (mouse monoclonal antibody, 1:250; Abcam, Cambridge, MA), CD45RO (mouse monoclonal antibody, 1:50; Novus, Littleton, NH), and FOXP3 (mouse monoclonal antibody, 1:100; Abcam). Staining results were evaluated by two independent pathologists who were blind to the clinical data pertaining to the patients For MMP9 and MMP11, samples with 0–5%, 6–25%, 26–50%, 51–75%, and >75% positive cells were scored as −, +, ++, +++, and ++++, respectively. Expression of CD45RO and FOXP3 was assessed by counting positive lymphocytes in five high-power fields and calculating their mean values.

Quality control

DNA preparation and PCR amplification was conducted at different locations within the laboratory, with specimens moving through the laboratory in only one direction. Environmental contamination was monitored consistently before each experiment, and laboratory areas were decontaminated regularly. To monitor potential contamination during DNA preparation, DNA was prepared from a colorectal tissue sample of a nude mouse after each batch of 6 pairs of human CRCs and adjacent NT samples. To confirm absence of contamination during DNA preparation, human β-actin and nusG gene of *F. nucleatum* were tested in these control samples.

Each 96-well plate used for real time-PCR contained several control samples, including three nude mouse colorectal DNA samples, processed along with the human specimens, as DNA preparation controls; four samples from which DNA template had been omitted; and, as positive controls, plasmids containing 10^9~10^2 copies of nusG gene DNA.

Statistical analysis

The difference in infection rates between tumor and matched NT were analyzed using the Chi-square test, whereas the difference in copy number was analyzed using the rank sum test. Univariate and multivariate logistic regression analyses were performed to estimate the odds ratios (ORs) and corresponding 95% confidence intervals (CIs) for associations between the presence of *F. nucleatum* DNA and clinicopathological features. CD45RO+ lymphocyte counts in normal and tumor tissues were compared using paired-samples *t*-tests, CD45RO+ lymphocyte counts in the high and low *F. nucleatum* copy groups were compared using independent-samples *t*-tests, and FOXP3+ lymphocyte counts were compared using the rank sum test. Expression of MMP9 and MMP11 in normal and carcinoma tissues, and in high and low-copy groups, was compared using Chi-squared tests. OS was analyzed using the Kaplan-Meier model and compared using the log-rank test. All statistical analyses were performed using SPSS 16.0 statistical software (SPSS, Chicago, IL, USA), with P<0.05 considered statistically significant.

Results

Infection rate and infection load of F. nucleatum infection in CRC patients were higher than in matched NT

F. nucleatum was detected in 118 of the 152 (77.6%) tumor tissues and in 87 of the 152 (57.2%) matched NT (P<0.001). Infection load (copy numbers of infection) ranged from 1 to 240,418 (median, 87) in the CRC tissues and from 2 to 3,685 (median, 37) in adjacent NT (P<0.001). In 84 of 152 patients (55.3%), *F. nucleatum* was detected in both the tumor and adjacent NT, with median copy numbers of 173 and 38, respectively. In 68 of these 84 patients, the copy number was higher in tumor tissues than in matched NT (P<0.001; *Table 2*).

Table 2 Association of F. nucleatum infection with clinicopathological features

Clinicopathological features	Tumor infection			Normal infection		
	Positive (%)	Negative (%)	P	Positive (%)	Negative (%)	P
Gender			NS			NS
Male	75 (63.6)	20 (36.4)		57 (60.0)	38 (40.0)	
Female	43 (75.4)	14 (24.6)		30 (52.6)	27 (47.4)	
Age, years			NS			NS
<60	57 (82.6)	12 (17.4)		40 (57.1)	30 (42.9)	
≥60	61 (73.5)	22 (26.5)		47 (57.3)	35 (42.7)	
Tumor site			NS			NS
Right-sided	42 (85.7)	7 (14.3)		36 (73.5)	13 (26.5)	
Left-sided	40 (75.5)	13 (24.5)		31 (58.5)	22 (41.5)	
Rectum	36 (72.0)	14 (28.0)		20 (40.0)	30 (60.0)	
Differentiation			<0.001			NS
Well	5 (14.3)	2 (28.6)		4 (57.1)	3 (42.9)	
Moderate	89 (74.8)	30 (25.2)		68 (57.1)	51 (42.9)	
Poor	23 (88.5)	3 (11.5)		15 (57.7)	11 (42.3)	
Depth of invasion			<0.001			NS
I	2 (33.3)	4 (66.7)		1 (16.7)	5 (83.3)	
II	16 (76.2)	5 (23.8)		10 (47.6)	11 (52.3)	
III	43 (76.8)	13 (23.2)		36 (64.3)	20 (35.7)	
IV	57 (82.6)	12 (17.4)		40 (58.0)	29 (42.0)	
Lymph node metastasis			0.010			NS
N0	47 (72.3)	18 (27.7)		39 (60.0)	26 (40.0)	
N1	36 (78.3)	10 (21.7)		27 (58.7)	19 (41.3)	
N2	32 (84.2)	6 (15.8)		19 (50.0)	19 (50.0)	
N3	3 (100.0)	0 (0.0)		2 (66.7)	1 (33.3)	
Distant metastasis			0.001			NS
M0	97 (77.0)	29 (23.0)		76 (60.3)	50 (39.7)	
M1	21 (80.8)	5 (19.2)		11 (42.3)	15 (57.7)	
Vascular invasion			NS			NS
Absent	86 (76.1)	27 (23.9)		65 (57.5)	48 (42.5)	
Present	32 (82.1)	7 (17.9)		22 (56.4)	17 (43.6)	
TNM stage			0.034			0.007
I	4 (66.7)	2 (33.3)		2 (33.3)	4 (66.7)	
II	41 (71.9)	16 (28.1)		36 (63.2)	21 (36.8)	
III	53 (82.8)	11 (17.2)		38 (59.4)	26 (40.6)	
IV	20 (80.0)	5 (20.0)		11 (44.0)	14 (56.0)	

NS, not significant.

Correlation of F. nucleatum infection with clinicopathological features

F. nucleatum infection of tumor tissue was related with poorer tumor differentiation (P<0.001), advanced invasion depth (P<0.001), lymph node metastasis (p=0.01), distant metastasis (P=0.001) and TNM stage (P=0.034), whereas infection of NT was only associated with TNM stage (P=0.007). For detailed data, please see *Table 2*. Using the median number of infectious particles in tumor tissue samples, we classified the 84 pairs of samples in which both tumor tissue and matched NT were positive for *F. nucleatum* into high- and low-abundance group. Then we analyzed the correlation between *F. nucleatum* copy number and patients' clinicopathological features and found that high-copy infection was associated only with tumor stage (P=0.023).

Immunohistochemical evaluation

In order to explore whether *F. nucleatum* infection can affect the biological behavior of host cells and tumor cells, we assayed MMP9, MMP11, CD45RO and FOXP3 expression in 16 pairs of T3N0M0 tumor and matched NT that were both infected with *F. nucleatum*. Expression of MMP9 was observed in the nucleus and the cytoplasm. MMP11 showed weak to moderate cytoplasmic staining, and extracellular material was often distinctly stained. CD45RO was observed in the cytoplasm or on the plasmalemma of lymphocytes, and FOXP3 was observed in the nucleus of lymphocytes (*Figure 1*). MMP9, MMP11 and FOXP3 were highly expressed in tumor tissues, and CD45RO was highly expressed in NT (*Table 3*). When we classified these 16 pairs of samples according to the median number of *F. nucleatum* copies in the tumor samples, we found that the mean number of lymphocytes positive for CD45RO was significantly higher in the low-copy group than in the high-copy group (120 *vs*. 81, P=0.001), whereas the mean number of lymphocytes positive for FOXP3 was significantly higher in the high-copy than in the low-copy group (54 *vs*. 3, P=0.001; *Figure 2*).

Survival analysis

Using the median number [87] of *F. nucleatum* copies in tumor tissues as a cut off value, we classified all 118 patients positive for tumor infection into a high- and a low-copy group, and compared OS after surgery in these two groups. Of the 59 patients in the low-copy group, 14 died, and median OS was 39.5 months. Of the 57 patients in the high-copy group, 24 died, and the median OS was 32.7 months.

The remaining 2 patients were lost to follow-up and the rate was 1.7%. Kaplan-Meier survival analysis and Log-rank test showed that patients in the high-copy group had a shorter postoperative survival than the low-copy group (P=0.027, *Figure 3*).

Next, we included other clinicopathological factors in the Cox model for multivariate survival analysis. In the total patients, we have found that tumor stage was an independent prognostic factor of postoperative survival in these CRC patients, that is, advanced stage was correlated poorer prognosis (P=0.018), however, other factors (including *F. nucleatum* infection) were not independent prognostic factors. Furthermore, we evaluated the effect of various factors on postoperative survival in the subset of CRC patients with *F. nucleatum* infection, and found that tumor stage and *F. nucleatum* infection were independent prognostic factors, that is, advanced stage and high-load infection were correlated poorer prognosis (P=0.023 and 0.032, respectively).

Discussion

Infection with microorganisms may play an important role in tumor development and progression. For example, HPV is involved in cervical cancer (16), EBV in nasopharyngeal carcinoma (17), *Helicobacter pylori* in gastric cancer (18), and HBV in hepatocellular carcinoma (19). *F. nucleatum* was detected in the lamina propria of tumor tissues in CRC patients, wherein infection rate and infection load was higher in tumors than in adjacent NT (6,7). These findings suggested that *F. nuleatum* may be closely associated with CRCs (20). Since these previous studies involved western patients, we assessed the infection rate and infection load of *F. nucleatum* in CRC patients in China, and evaluated the potential effect of this bacterium on the hosts.

Using strict inclusion criteria, we chose 152 CRC patients with complete clinical and pathological data who underwent radical resection. Using quantitative PCR, we determined the copy number of *F. nucleatum* DNA in samples and found that both *F. nucleatum* infection rate and copy number were significantly higher in tumor tissues than in adjacent NT (6,7). We also assessed the association of *F. nucleatum* infection with clinicopathological features and found that *F. nucleatum* infection of tumor tissue was related with poorer tumor differentiation (P<0.001), advanced invasion depth (P<0.001), lymph node metastasis (P=0.01), distant metastasis (P=0.001) and TNM stage (P=0.034). depth of tumor invasion (P=0.039), whereas infection of NT was only associated with TNM stage (P=0.007). In addition, when we

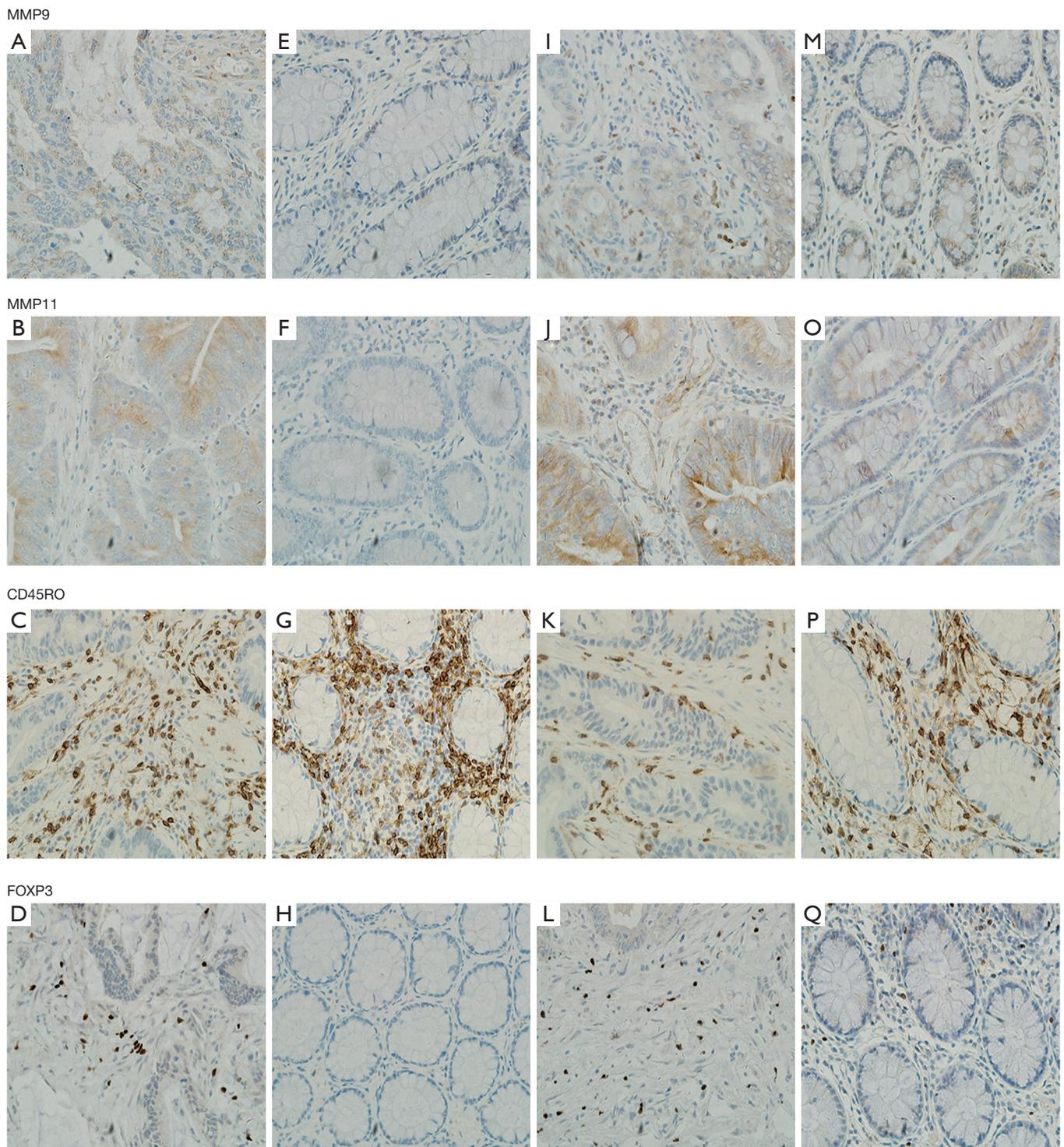MMP9

MMP11

CD45RO

FOXP3

Figure 1 Immunohistochemistry staining in 16 pairs of CRC patients. (A-D) are MMP9, MMP11, CD45RO and FOXP3 staining in low-abundance colorectal cancer tissues; (E-H) are MMP9, MMP11, CD45RO and FOXP3 staining in the corresponding normal tissue; (I-L) are MMP9, MMP11, CD45RO and FOXP3 staining in high-abundance colorectal cancer tissues; (M-Q) are MMP9, MMP11, CD45RO and FOXP3 staining in the corresponding normal tissue. All pictures were taken using an optical microscope, at 400× magnification.

Table 3 Immunohistochemical expression of MMP9 and MMP11 in 16 pairs of CRC tumor tissues and matched normal tissue, assorted by high and low abundance of *F. nucleatum* copies

Staining strength	Normal tissue			Tumor tissue			P[†]
	High-load	Low-load	P	High-load	Low-load	P	
MMP9 (%)			–			0.149	0.001
–	9 (100.0)	7 (100.0)		4 (44.4)	3 (42.9)		
+/++	0 (0.0)	0 (0.0)		5 (55.6)	4 (57.1)		
MMP11 (%)			0.603			0.622	0.009
–	8(88.9)	6(85.7)		5 (55.6)	5 (71.4)		
+/++	1(11.1)	1(14.3)		4 (44.4)	2 (28.6)		

[†], comparison between tumors and matched normal tissue samples.
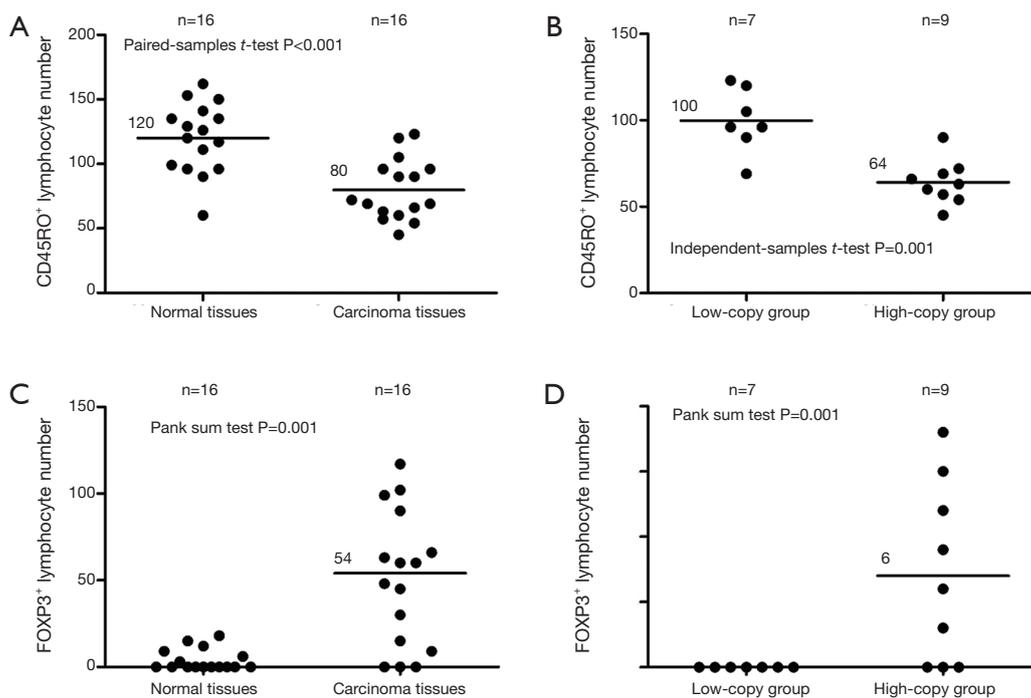
Figure 2 Comparison of CD45RO^{+} (A,B) and FOXP3^{+} (C,D) lymphocyte count in 16 pairs CRC patients. (A) Comparison of CD45RO^{+} lymphocyte count in 16 pairs of CRC patients between tumor tissues and matched normal tissues using paired sample *t*-test; (B) comparison of CD45RO^{+} lymphocyte count between high-abundance and low-abundance groups using independent sample *t*-test; (C) comparison of FOXP3^{+} lymphocyte count in 16 pairs of CRC patients between tumor tissues and matched normal tissues using rank sum test; (D) comparison of FOXP3^{+} lymphocyte count between high-abundance and low-abundance groups using rank sum test.

compared the clinicopathological features in patients with high- and low-copy numbers of *F. nucleatum*, we found that high-copy infection was only correlated with tumor stage (P=0.023). Previous studies also have shown correlations of *F. nucleatum* abundance with patient geographic location (7) and number of metastatic lymph nodes (6). Survival analysis showed that patients in the high-copy group had shorter survival after surgery than the low-copy group (P=0.027). These findings suggested that *F. nucleatum* might play a role in progression and prognosis of CRC patients.

F. nucleatum can stimulate locally infected epithelial cells to secrete MMPs and promote the apoptosis of immune cells
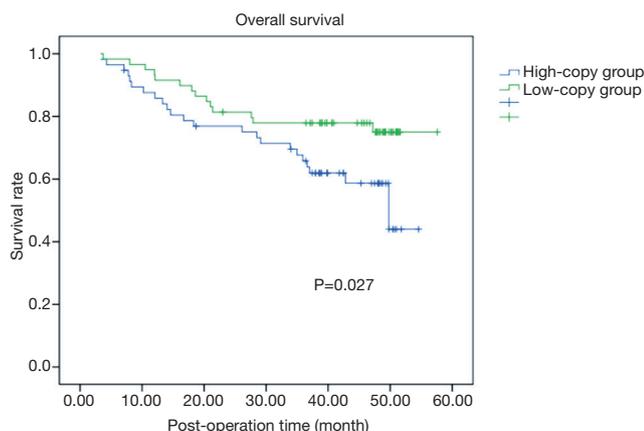
Figure 3 Kaplan-Meier overall survival analysis of colorectal cancer patients. We classified all 118 patients positive for tumor infection into a high- and a low-copy group, of the 59 patients in the low-copy group, 14 died, and median OS after surgery was 39.5 months. Of the 57 patients in the high-copy group, 24 died, and the median OS after surgery was 32.7 months (P=0.027).

(4). In our study, these characteristics were assessed in CRC patients by immunohistochemical assays of MMP9, MMP11, CD45RO and FOXP3 in tumor tissues and corresponding NT. To minimize confounding factors, we selected 16 patients of T3N0M0 tumor staging, with both tumor and matched NT being infected with *F. nucleatum*. We found that, compared with specimens with low infection-load, those with high infection-load contained fewer CD45RO⁺ cytotoxic T lymphocytes (21), which can secrete cytokines involved in immune function and kill viruses and tumor cells, thus constituting an important defense in anti-viral and anti-tumor immunity. Therefore, a reduction in the number of CD45RO⁺ may reflect an inactive local immune status. In contrast, specimens in the high-abundance group contained more T regulatory lymphocytes, as determined by FOXP3⁺ expression (21), than the low-abundance group. These findings also indicated that localized *F. nucleatum* infection in CRC patients may play a role in immune suppression. There was no difference in MMP9 and MMP11 expression between these two groups, which might be due to the complex mechanism by which the secretion of MMPs is regulated. Moreover, the relatively small number of patient sample assayed immunohistochemically was insufficient to rule out confounding factors.

The mechanisms by which *F. nucleatum* caused CRC have been elucidated in recent years. *F. nucleatum* can generate a proinflammatory microenvironment that is conducive for colorectal neoplasia progression through recruitment of tumor-infiltrating immune cells (22). In addition, another study also demonstrated that *F. nucleatum* can adhere to, invade, and induce oncogenic and inflammatory responses to stimulate growth of CRC cells through its unique FadA adhesin (23). So in future study, we will also evaluate the expression level of FadA adhesin, and also will explore the possible correlation of FadA adhesin with other markers of CRC or with clinicopathological factors and prognosis of CRC patients.

To our knowledge, this is the first study to assess the effect of *F. nucleatum* infection in CRC patients in Chian, both at the systemic and local levels. We used ATCC 25,586 type-specific primers for F.N. q-PCR, which might have underestimated the total infection rate. Another limitation was the lack of normal colorectal mucosa specimens from healthy individuals, and this may be remedied by cooperating with our endoscopic department in the future.

Conclusions

Our results have provided evidence that *F. nucleatum* may act as a pathogenic factor in CRC patients, which may lead to improvement in the prevention, diagnosis and treatment of CRC. *F. nucleatum* infection was correlated with tumor metastasis and postoperative survival of CRC patients.

Acknowledgments

Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2016.10.45). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the

institutional review board of Peking University Cancer Hospital (Ethical approval number: 2008110621), and written informed consent was acquired from each patient.

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