Prognostic and clinicopathological significance of *Fusobacterium nucleatum* in colorectal cancer: a systemic review and meta-analysis

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Background: *Fusobacterium nucleatum* has been identified to promote tumor progression in colorectal cancer (CRC). However, association between *F. nucleatum* and prognostic or clinicopathological features has been diverse among studies, which could be affected by type of biospecimen (formalin-fixed paraffin-embedded or fresh frozen [FF]). **Methods:** Articles were systemically reviewed for studies that included the correlation between *F. nucleatum* and prognosis or clinicopathological features in CRC. **Results:** Ten articles, eight studies with survival-related features involving 3,199 patients and nine studies with clinical features involving 2,655 patients, were eligible for the meta-analysis. Overall survival, disease-free survival, and cancer-specific survival were all associated with worse prognosis in *F. nucleatum*–high patients (p < .05). In subgroup analysis, only studies with FF tissues retained prognostic significance with *F. nucleatum.* In meta-analysis of clinicopathological variables, *F. nucleatum* level was associated with location within colon, pT category, *MLH1* hypermethylation, microsatellite instability status, and *BRAF* mutation regardless of type of biospecimen. However, lymph node metastasis and *KRAS* mutation was only associated with *F. nucleatum* level in FF-based studies. **Conclusions:** In conclusion, type of biospecimen could affect the role of *F. nucleatum* as a biomarker associated with clinicopathological features and prognosis.

Key Words: Fusobacteria; Colorectal neoplasms; Prognosis

In recent years, it has been established that gut microbiota is closely linked to various intestinal human disease including colorectal cancer (CRC) via microbiome dysbiosis [1-3]. Among the heterogeneous microbiota, *Fusobacterium nucleatum*, an anaerobic, gram-negative bacteria, has been enriched in CRC tumor tissues and stool specimen compared with normal tissues [4,5]. Abundance of *F. nucleatum* is gradually increased from normal to precancerous adenomatous lesions to carcinoma in CRC and suggests a significant role of *F. nucleatum* in tumor progression of CRC [6,7].

F. nucleatum has various mechanisms that influence carcinogenesis which includes promoting of E-cadherin/β-catenin signaling via adhesion protein FadA [8], adhering to cancer cells, colonizing, and inhibiting immunity by leptin Fap2 [2,9], inducing development of CRC by mircoRNA-21–mediated path-

way [10], and increasing chemoresistance via autophagy [11,12]. Recently, it has been reported that *F. nucleatum* is positive in CRC cells in liver metastases, which suggests that *F. nucleatum* may also act as an integral component in CRC metastasis [13]. Furthermore, in xenograft tumor model, treating *F. nucleatum* with antibiotics resulted in reduction of tumor growth.

Even though *F. nucleatum* plays a key role in CRC progression and metastasis, studies that report the characteristics of *F. nucleatum* in prognosis and clinicopatholigcal features by using CRC tissues, nevertheless, has shown conflicting results. Several studies considered *F. nucleatum* level as a suitable biomarker for worse survival [14-16] in CRC patients while other studies did not detect correlation between *F. nucleatum* level and prognosis in overall CRC patients [17,18]. Similar discrepancies between studies arise from other clinicopathological studies such as *KRAS* mutation,

tumor location, and microsatellite instability (MSI) status. Although the reason for discordance could be due to heterogeneity in CRC patient populations [19], this could also be affected by the type of biospecimen used for each study, namely formalin-fixed paraffin-embedded (FFPE) tissue or fresh frozen (FF) tissue. A recent article addresses differences between paired FFPE and FF samples when detecting *F. nucleatum* in CRC [20], but a systemic review that discuss the impact of biospecimen in detecting *F. nucleatum* has not been available.

In this study, we conducted a systemic review and meta-analysis to determine the prognostic and clinicopathological significance of *F. nucleatum* in CRC. Our aim is to clarify how the selection of biospecimen could allocate different roles of microbiome of CRC by affecting the association between *F. nucleatum* level and survival or clinicopathological features.

MATERIALS AND METHODS

Literature search strategy

Articles relevant with the subject were retrieved from electronic databases, PubMed, Embase, and Web of Science until December 3 of 2020. Search terms were, "Fusobacterium nucleatum, Fusobacterium, Fusobacteria, or *F. nucleatum*" and "colorectal cancer, colon cancer, rectal cancer, colorectal carcinoma, or CRC". Each article was examined carefully to determine studies with identical patient population. When multiple studies present overlapping patient data, we selected the studies with the largest patient cohort for clinical data and survival data, respectively.

Inclusion and exclusion criteria

Studies that were eligible for the inclusion in this meta-analysis were as follows: (1) full length articles published in English that detected Fusobacterium nucleatum from human tumor samples derived from CRC tissue, (2) studies with CRC samples divided into high and low (or high and low/negative) according to *F. nucleatum* level, (3) studies that included clinicopathological, molecular, or survival-associated data that were correlated with *F. nucleatum* level. Studies that only contain abstract or posters, studies could not be arranged as dichotomized *F. nucleatum* level data, studies with insufficient data, studies that used more than one type of biospecimen to detect *F. nucleatum* level, and studies that detected *F. nucleatum* level in stools were excluded.

Data extraction and quality assessment

Two reviewers (Y.K. and G.H.K.) independently collected data from eligible articles according to the criteria. Newcastle-Ottawa Scale was used to evaluate study quality. A score higher than six out of nine criteria was defined as qualified. Name of author, year of publication, number of total patients, number of *F. nucleatum* positive patients, type of biospeicmen (FFPE or FF), method of *F. nucleatum* level detection, survival data, and clinicopathological data associated with *F. nucleatum* level were collected from each study. Survival data were extracted as hazard ratio [HR] and 95% confidence intervals [CIs] from univariate Cox analysis. When only Kaplan-Meier curves were presented for survival analysis, HR and 95% CIs were estimated by using Engauge Digitizer V9.8 (http://digitizer.sourceforge.net) and spreadsheets provided by Tierney et al. [21]. When a three-tier survival was given (negative, low, and high) we pooled negative and low as reference to calculate HR and 95% CIs for high and rearranged it into two-tier (low/negative and high).

Statistical analysis

Meta-analysis was conducted using R software ver. 3.5.3 (https://cran.r-project.org/). Pooled HR and 95% CIs for overall survival (OS), disease-free survival (DFS), and cancer-specific survival (CSS) and pooled odds ratio and 95% CIs for clinicopathological features were analyzed via R package 'meta' and 'dmetar'. Subgroup analysis was performed if multiple types of biospecimen existed within a pooled analysis. Statistic heterogeneity was determined by Cochran's Q and I^2. When heterogeneity was observed ($p < .05$ or $I^2 > 50\%$) random effect model was used for the analysis instead of fixed effect model. A graphical funnel plot was used to evaluate publication bias.

RESULTS

Literature search and study characteristics

Four hundred and thirty-two records were collected from PubMed, Embase, and Web of Science (Fig. 1). After removing 76 duplications, 356 records were screened by abstract and 32 records were categorized as being relevant with the subject. These records were reviewed in detail and 10 articles, eight studies with survival-related features involving 3,199 patients [14-18,22-24] and nine studies with clinical features involving 2,655 patients [14-17,22-25], were selected eligible for the meta-analysis (Table 1). Seven studies were included in the meta-analysis for both survival-related and clinical features [14-17,22-24]. Two studies had overlapping patients but were used for meta-analysis of either clinical or survival-related features, respectively [18,25]. Six out of the 10 articles assessed *F. nucleatum* level with FFPE tissues [6,16-18,24,25] and four articles used FF tissues

to detect *F. nucleatum* [14,15,22,23].

Meta-analysis between *F. nucleatum* level and survival

The meta-analysis between *F. nucleatum* level and prognostic values of OS (Fig. 2A), DFS (Fig. 2B), and CSS (Fig. 2C) in CRC has shown that high *F. nucleatum* level is associated with poor OS, DFS, and CSS (OS: HR, 1.58; 95% CI, 1.28 to 1.94; p < .001; DFS: HR, 1.76; 95% CI, 1.06 to 2.93; p = .030; CSS: HR, 1.72; 95% CI, 1.05 to 2.83; p = .031, respectively). In subgroup anal-
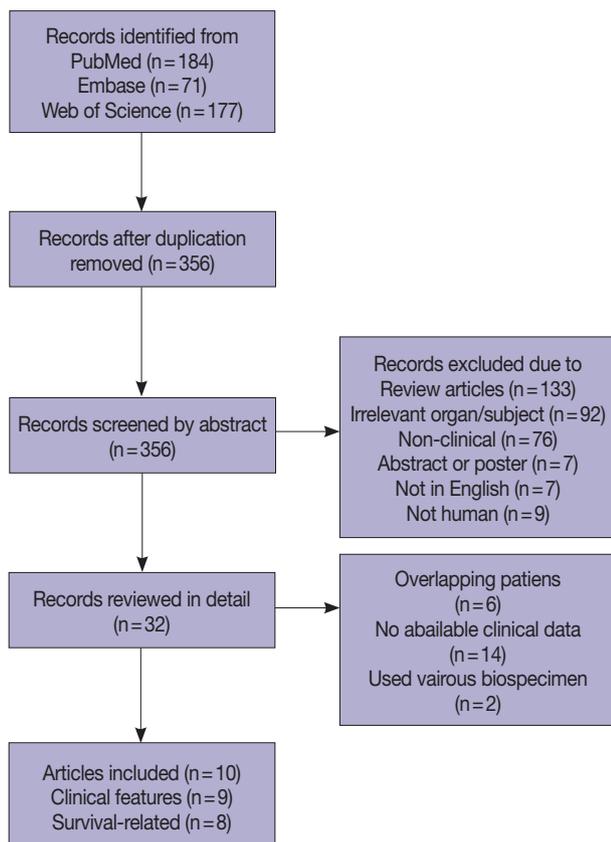
ysis, studies with FF samples (p < .001), but not those with FFPE samples (p = .168) retained prognostic significance between *F. nucleatum* level and OS (Table 2, Supplementary Fig. S1). Furthermore, studies with FFPE samples did not show significant correlation between *F. nucleatum* level and DFS (p = .214). Analyzing studies with stage I though IV CRC did not affect prognostic significance between *F. nucleatum* level and OS (HR, 1.41; 95% CI, 1.11 to 1.80; p = .005).

Meta-analysis between *F. nucleatum* level and clinicopathological features

Meta-analysis for correlation between *F. nucleatum* level and clinicopathological features of CRC are assessed in Table 3. High *F. nucleatum* level was associated with proximal colon location (p < .001), worse tumor differentiation (p = .002), high pT category (p < .001), presence of distal metastasis (p = .007), *MLH1* hypermethylation (p < .001), and MSI-high CRC (p = .001) when all studies were considered. In subgroup analysis (Table 4), studies with FFPE samples showed significant correlation between high *F. nucleatum* level and proximal colon location (p = .001), worse tumor differentiation (p = .015), high pT category (p < .001), *MLH1* hypermethylation (p < .001), CpG island methylator phenotype (CIMP)–high status (p < .001), and MSI-high status (p < .001). Studies with FF samples, on the other hand, demonstrated that high *F. nucleatum* level is significantly correlated with high pT category (p = .001), presence of lymph node metastasis (p = .001), and presence of *KRAS* mutation (p = .007), which showed borderline significance with high *F. nucleatum* level in overall studies (p = .052).

Publication bias

Begg's funnel plot was evaluated for presence of potential publication bias in meta-analysis associated with survival (Fig. 3). Visible asymmetry was observed with Egger's test reporting p-

Fig. 1. Flow chart of literature search and study selection.

Table 1. Characteristics of articles included in the meta-analysis

Authors	Year	No. of patients	No. of positives	Biospecimen	Method	Survival	Clinical	Stage
Yan et al. [16]	2017	280	187	FFPE	qPCR	O	O	III/IV
Wei et al. [14]	2016	180	90	FF	qPCR	O	O	I–III
Yamaoka et al. [15]	2018	100	50	FF	ddPCR	O	O	I–IV
Samkamoto et al. [18]	2018	1,069	67	FFPE	qPCR	O		I–IV
Sun et al. [22]	2016	152	59	FF	qPCR	O	O	I–IV
Kunzmann et al. [23]	2019	190	61	FF	qPCR	O	O	I–IV
Oh et al. [17]	2019	593	204	FFPE	qPCR	O	O	II/III
Chen et al. [24]	2019	91	25	FFPE	qPCR	O	O	II/III
Ito et al. [6]	2015	511	143	FFPE	qPCR		O	I–IV
Mima et al. [25]	2015	1,102	69	FFPE	qPCR		O	I–IV

FFPE, formalin-fixed paraffin-embedded; qPCR, quantitative polymerase chain reaction; FF, fresh frozen; ddPCR, droplet digital polymerase chain reaction.
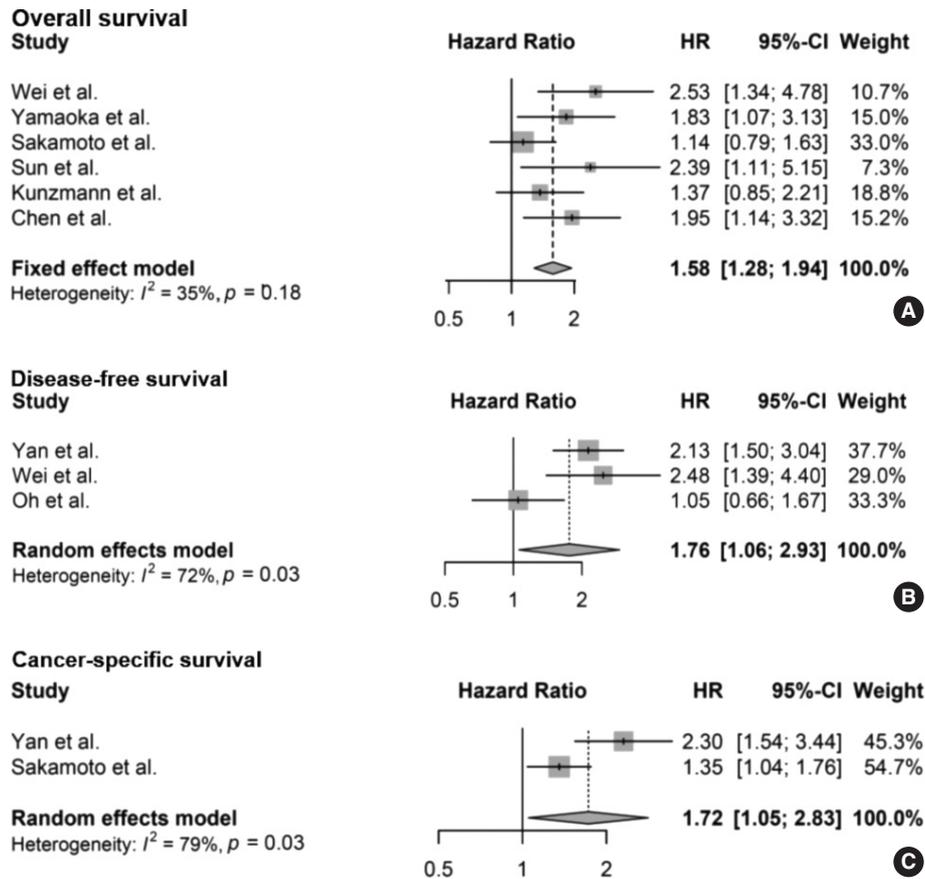
Fig. 2. Forest plot for the correlation between *Fusarium nucleatum* and survival in colorectal cancer [14-18,22-24]: (A) overall survival, (B) disease-free survival, and (C) cancer-specific survival. HR, hazard ratio; CI, confidence interval.

Table 2. Subgroup analysis associated with survival

Survival	Specimen	Study	HR	95% CI	p-value
Overall survival	FFPE	2	1.43	0.85–2.42	.168
	FF	4	1.84	1.36–2.48	<.001
Disease-free survival	FFPE	2	1.52	0.76–3.04	.214

HR, hazard ratio; CI, confidence interval; FFPE, formalin-fixed paraffin-embedded; FF, fresh frozen.

value of .006 for OS but not for DFS (p = .935).

DISCUSSION

F. nucleatum has been associated with cancer development and progression of CRC, as well as a potential target of cancer treatment. *F. nucleatum* is abdundant in CRC tissues from patients with metastasis than those from non-metastatic CRC patients [26]. Presence *F. nucleatum* was essential in establishment of CRC patients-derived xenografts in mice [13]. Not only does infection of *F. nucleatum* has promoted metastasis in CRC cells via autophage but also is found in distant metastasis sites including liver, lung, and lymph nodes, although the mechanism is yet to be un-

veiled [9,13,26,27]. Moreover, previous studies have suggested that *F. nucleatum* provoke chemoresistance inhibiting caspase cascade and activating autophagy pathway to prevent apoptosis in CRC cells [11,28].

It is still unclear if *F. nucleatum* is a significant driver of tumor initiation and *F. nucleatum* level detected from CRC tissues and association with prognosis and clinicopathological variables has not been consistent among studies. Herein, we hypothesized that this could be partially caused by different type of biospecimen being used for each study and investigated its affect by a systemic review and meta-analysis with the difference between FFPE and FF into consideration.

High *F. nucleatum* level was associated with worse survival in

OS, DFS, and CSS in overall meta-analysis. Subgroup analysis revealed that studies with FF show a significant correlation between *F. nucleatum* level and OS. However, studies with FFPE tissues show that a significant correlation between *F. nucleatum* and OS or DFS is not observed.

Association between *F. nucleatum* level and clinicopathological features are much more diverse. A pooled analysis of FFPE and FF studies together shows that high *F. nucleatum* level samples

Table 3. Meta-analysis associated with clinical features

Clinical feature	Study	OR	95% CI	p-value
Age	5	0.993	0.787–1.254	.954
Sex (female/male)	9	1.087	0.910–1.299	.356
Proximal/Distal colon	4	1.642	1.252–2.153	<.001
Rectum/Colon	5	1.184	0.899–1.560	.229
Tumor size	2	0.897	0.592–1.360	.609
Differentiation	7	1.547	1.166–2.052	.002
Vascular invasion	2	0.627	0.337–1.167	.141
Perineural invasion (FFPE only)	2	1.022	0.722–1.448	.900
pT category	5	2.253	1.633–3.108	<.001
Lymph node metastasis	5	1.308	0.979–1.748	.069
Distant metastasis	4	1.912	1.196–3.058	.007
Stage	6	1.110	0.885–1.394	.367
CIMP status (FFPE only)	3	2.612	1.849–3.690	<.001
MLH1 hypermethylation	3	2.852	1.987–4.095	<.001
MSI status	5	2.982	2.175–4.086	<.001
KRAS mutation	5	1.248	0.998–1.561	.052
BRAF mutation	4	1.869	1.273–2.745	.001
PIK3CA mutation	3	1.440	0.965–2.147	.074

OR, odds ratio; CI, confidence interval; FFPE, formalin-fixed paraffin-embedded; CIMP, CpG island methylator phenotype; MSI, microsatellite instability.

Table 4. Subgroup analysis for clinical features

Clinical feature	Specimen	Study	OR	95% CI	p-value
Age	FFPE	2	1.158	0.871–1.539	.312
	FF	3	0.720	0.478–1.086	.117
Sex	FFPE	5	1.096	0.891–1.349	.386
	FF	4	1.063	0.753–1.501	.727
Proximal/Distal colon	FFPE	2	1.676	1.220–2.303	.001
	FF	2	1.554	0.925–2.612	.096
Rectum/Colon	FFPE	2	1.259	0.841–1.884	.263
	FF	3	1.120	0.767–1.636	.557
Differentiation	FFPE	4	1.513	1.083–2.112	.015
	FF	3	1.632	0.958–2.778	.071
pT category	FFPE	2	2.187	1.437–3.329	<.001
	FF	3	2.354	1.428–3.880	.001
Lymph node metastasis	FFPE	2	0.832	0.549–1.262	.387
	FF	3	2.009	1.337–3.017	.001
Distant metastasis	FF	3	1.669	0.863–3.228	.128
Stage	FFPE	2	0.917	0.673–1.251	.586
	FF	4	1.389	0.993–1.943	.055
MLH1 hypermethylation	FFPE	2	3.210	2.208–4.667	<.001
MSI status	FFPE	4	2.757	1.987–3.824	<.001
KRAS mutation	FFPE	3	1.125	0.881–1.436	.345
	FF	2	2.220	1.246–3.954	.007
BRAF mutation	FFPE	3	1.882	1.260–2.811	.002
PIK3CA mutation	FFPE	2	1.464	0.960–2.233	.076

OR, odds ratio; CI, confidence interval; FFPE, formalin-fixed paraffin-embedded; FF, fresh frozen; MSI, microsatellite instability.
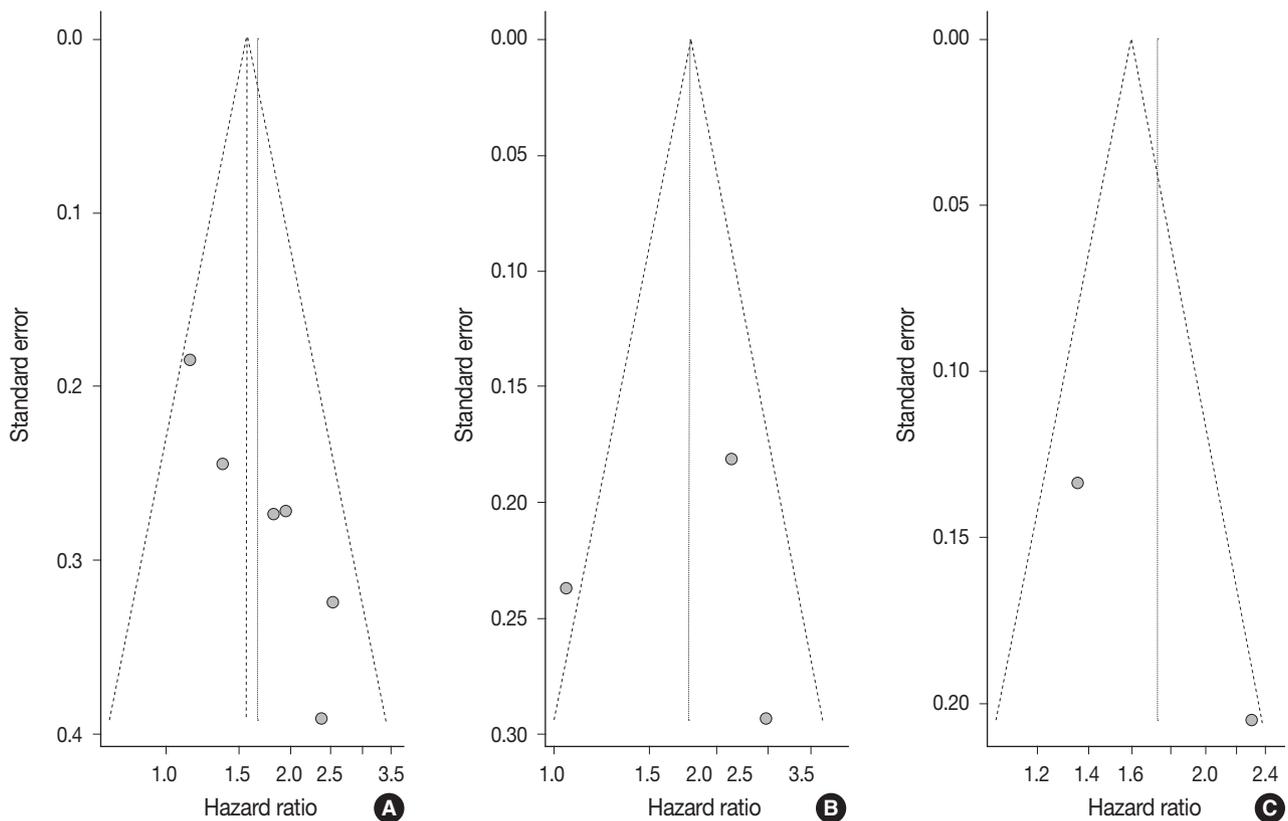
Fig. 3. Funnel plot for studies included in meta-analysis between *Fusarium nucleatum* level and survival: (A) overall survival, (B) disease-free survival, and (C) cancer-specific survival.

are significantly enriched in proximal colon, but on subgroup analysis, only studies using FFPE samples retain statistical significance for the same variable. Similar findings could be observed for correlation between *F. nucleatum* level and distant metastasis. On the contrary, pT category, *MLH1* hypermethylation, and MSI status remained its positive correlation with *F. nucleatum* level regardless of the biospecimen being used. Lymph node metastasis and *KRAS* mutation were only significant in FF tissues and *MLH1* hypermethylation was only significant in FFPE tissues but not in its amalgamated state. Interestingly, the aforementioned features are all considered as hallmark characteristics of MSI-high CRC [29]. These findings emphasize that regardless of type of specimen, high *F. nucleatum* level is strongly indicative of MSI-high status.

There are several putative explanations for discrepancy of results between biospecimen. Mean patient number for studies with FFPE tissue (515.4 patients per study) is more than three times larger than the mean patient number for studies with FF tissues (155.5 patients per study). On the contrary, *F. nucleatum* positivity is significantly higher in studies with FF tissues compared with those of FFPE tissue (41.8% and 24.4%. respectively) which may

be a result of a lower threshold in studies with FF tissues to compensate for smaller sample size. This may allude to the fact that features with relatively low incidence such as *KRAS* mutation, could have a higher chance of being categorized as high *F. nucleatum* level sample in studies with FF compared with studies with FFPE.

As an alternative explanation, lower *F. nucleatum* positivity in FFPE tissues may reflect the inherited nature of FFPE samples rather than inflation of threshold in studies with FF tissues. In a study that measured *F. nucleatum* level in paired FFPE and FF tissues of CRC, FF tissues had higher detection rate of overall *F. nucleatum* resulting in difference in survival and clinicopathological features from FFPE-measured samples [20]. Furthermore, concordance between *F. nucleatum* positive cases in FF and those in FFPE samples were low. Discrepancy between DNA results of FFPE and FF samples has also been reported in other paired tumor tissues [30]. This was in part due to inter-strand crosslink between complementary DNA strands which could be aggravated by prolonged fixation [31]. Other potential factors of low *F. nucleatum* detection in FFPE samples includes fragmentation and degeneration of DNA, paraffin embedding, and storage process

[25,32].

Recently, two articles analyzed the correlations between *F. nucleatum* level and prognosis in CRC with meta-analysis [33,34]. Both articles showed a significant correlation between *F. nucleatum* level and OS, but only one study showed a significant correlation between *F. nucleatum* level and DFS. Herein, we suggest that type of biospecimen that was included in each meta-analysis could have affected the discrepancy between the articles.

Limitation of our study is that we cannot identify pathological and molecular discrepancies between FF and FFPE tissues such as correlation between *F. nucleatum* level and T cell density, CIMP, and various immunohistochemical results due to lack of studies with FF that obtain related data. Moreover, although meta-analysis provides evidence that discordance between FF and FFPE samples are present in detecting *F. nucleatum*, we could not narrow down the cause of the misalignment between the two biospecimen.

In conclusion, *F. nucleatum* level is significantly associated with pathological and molecular features including pT category, *MLH1* hypermethylation, and MSI status regardless of biospecimen, but other survival, clinical, and molecular features, such as location of tumor within the colon, distant metastasis, and *KRAS* mutation, could be affected by type of biospecimen in CRC. Therefore, although a strong association between MSI and *F. nucleatum* is always evident, selection of biospecimen could affect the role of *F. nucleatum* as a biomarker in CRC to predict and monitor tumor progression and prognosis as well as evaluate treatment effect.

Supplementary Information

The Data Supplement is available with this article at https://doi.org/10.4132/jptm.2022.03.13.

Ethics Statement

Not applicable.

Availability of Data and Material

All data generated or analyzed during the study are included in this published article (and its supplementary information files).

Code Availability

Not applicable.

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Conflicts of Interest

The authors declare that they have no potential conflicts of interest.

References

1. Wu Y, Wu J, Chen T, et al. *Fusobacterium nucleatum* potentiates intestinal tumorigenesis in mice via a Toll-like receptor 4/p21-activated kinase 1 cascade. Dig Dis Sci 2018; 63: 1210-8.
2. Kostic AD, Chun E, Robertson L, et al. *Fusobacterium nucleatum* potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. Cell Host Microbe 2013; 14: 207-15.
3. Marchesi JR, Adams DH, Fava F, et al. The gut microbiota and host health: a new clinical frontier. Gut 2016; 65: 330-9.
4. Kostic AD, Gevers D, Pedamallu CS, et al. Genomic analysis identifies association of *Fusobacterium* with colorectal carcinoma. Genome Res 2012; 22: 292-8.
5. Castellarin M, Warren RL, Freeman JD, et al. *Fusobacterium nucleatum* infection is prevalent in human colorectal carcinoma. Genome Res 2012; 22: 299-306.
6. Ito M, Kanno S, Nosho K, et al. Association of *Fusobacterium nucleatum* with clinical and molecular features in colorectal serrated pathway. Int J Cancer 2015; 137: 1258-68.
7. McCoy AN, Araujo-Perez F, Azcarate-Peril A, Yeh JJ, Sandler RS, Keku TO. *Fusobacterium* is associated with colorectal adenomas. PLoS One 2013; 8: e53653.
8. Rubinstein MR, Wang X, Liu W, Hao Y, Cai G, Han YW. *Fusobacterium nucleatum* promotes colorectal carcinogenesis by modulating E-cadherin/beta-catenin signaling via its FadA adhesin. Cell Host Microbe 2013; 14: 195-206.
9. Abed J, Emgard JE, Zamir G, et al. Fap2 mediates *Fusobacterium nucleatum* colorectal adenocarcinoma enrichment by binding to tumor-expressed Gal-GalNAc. Cell Host Microbe 2016; 20: 215-25.
10. Yang Y, Weng W, Peng J, et al. *Fusobacterium nucleatum* increases proliferation of colorectal cancer cells and tumor development in mice by activating Toll-like receptor 4 signaling to nuclear factor-kappaB, and up-regulating expression of microRNA-21. Gastroenterology 2017; 152: 851-66.
11. Yu T, Guo F, Yu Y, et al. *Fusobacterium nucleatum* promotes chemoresistance to colorectal cancer by modulating autophagy. Cell 2017; 170: 548-63.
12. Mandip KC, Steer CJ. Novel mechanisms of chemoresistance by *Fusobacterium nucleatum* involve not so novel pathways of microRNAs and autophagy. Transl Cancer Res 2018; 7(Suppl 1): S10-5.
13. Bullman S, Pedamallu CS, Sicinska E, et al. Analysis of *Fusobacterium* persistence and antibiotic response in colorectal cancer. Science 2017; 358: 1443-8.
14. Wei Z, Cao S, Liu S, et al. Could gut microbiota serve as prognostic biomarker associated with colorectal cancer patients' survival? A pilot study on relevant mechanism. Oncotarget 2016; 7: 46158-72.
15. Yamaoka Y, Suehiro Y, Hashimoto S, et al. *Fusobacterium nucleatum* as a prognostic marker of colorectal cancer in a Japanese pop-

ulation. J Gastroenterol 2018; 53: 517-24.

16. Yan X, Liu L, Li H, Qin H, Sun Z. Clinical significance of *Fusobacterium nucleatum*, epithelial-mesenchymal transition, and cancer stem cell markers in stage III/IV colorectal cancer patients. Onco Targets Ther 2017; 10: 5031-46.

17. Oh HJ, Kim JH, Bae JM, Kim HJ, Cho NY, Kang GH. Prognostic impact of *Fusobacterium nucleatum* depends on combined tumor location and microsatellite instability status in stage II/III colorectal cancers treated with adjuvant chemotherapy. J Pathol Transl Med 2019; 53: 40-9.

18. Sakamoto Y, Mima K, Daitoku N, et al. *Fusobacterium nucleatum* in colorectal cancer liver metastasis and patient prognosis. Cancer Sci 2018; 109: 1360.

19. Chernyavskiy P, Kennerley VM, Jemal A, Little MP, Rosenberg PS. Heterogeneity of colon and rectum cancer incidence across 612 SEER counties, 2000-2014. Int J Cancer 2019; 144: 1786-95.

20. de Carvalho AC, de Mattos Pereira L, Datorre JG, et al. Microbiota profile and impact of *Fusobacterium nucleatum* in colorectal cancer patients of Barretos Cancer Hospital. Front Oncol 2019; 9: 813.

21. Tierney JF, Stewart LA, Ghersi D, Burdett S, Sydes MR. Practical methods for incorporating summary time-to-event data into meta-analysis. Trials 2007; 8: 16.

22. Sun Y, An QM, Tian XY, et al. *Fusobacterium nucleatum* infection is correlated with tumor metastasis and postoperative survival of colorectal cancer patients in China. Transl Cancer Res 2016; 5: 579-88.

23. Kunzmann AT, Proenca MA, Jordao HW, et al. *Fusobacterium nucleatum* tumor DNA levels are associated with survival in colorectal cancer patients. Eur J Clin Microbiol Infect Dis 2019; 38: 1891-9.

24. Chen Y, Lu Y, Ke Y, Li Y. Prognostic impact of the *Fusobacterium nucleatum* status in colorectal cancers. Medicine (Baltimore) 2019; 98: e17221.

25. Mima K, Sukawa Y, Nishihara R, et al. *Fusobacterium nucleatum* and T cells in colorectal carcinoma. JAMA Oncol 2015; 1: 653-61.

26. Chen Y, Chen Y, Zhang J, et al. Fusobacterium nucleatum promotes metastasis in colorectal cancer by activating autophagy signaling via the upregulation of CARD3 expression. Theranostics 2020; 10: 323-39.

27. Wang S, Liu Y, Li J, et al. *Fusobacterium nucleatum* acts as a pro-carcinogenic bacterium in colorectal cancer: from association to causality. Front Cell Dev Biol 2021; 9: 710165.

28. Zhang S, Yang Y, Weng W, et al. *Fusobacterium nucleatum* promotes chemoresistance to 5-fluorouracil by upregulation of BIRC3 expression in colorectal cancer. J Exp Clin Cancer Res 2019; 38: 14.

29. Bae JM, Kim JH, Kang GH. Molecular subtypes of colorectal cancer and their clinicopathologic features, with an emphasis on the serrated neoplasia pathway. Arch Pathol Lab Med 2016; 140: 406-12.

30. Wen X, Jeong S, Kim Y, et al. Improved results of LINE-1 methylation analysis in formalin-fixed, paraffin-embedded tissues with the application of a heating step during the DNA extraction process. Clin Epigenetics 2017; 9: 1.

31. Freifelder D, Davison PF. Physicochemical studies on the reaction between formaldehyde and DNA. Biophys J 1963; 3: 49-63.

32. Imrit K, Goldfischer M, Wang J, et al. Identification of bacteria in formalin-fixed, paraffin-embedded heart valve tissue via 16S rRNA gene nucleotide sequencing. J Clin Microbiol 2006; 44: 2609-11.

33. Gethings-Behncke C, Coleman HG, Jordao HWT, et al. *Fusobacterium nucleatum* in the colorectum and its association with cancer risk and survival: a systematic review and meta-analysis. Cancer Epidemiol Biomarkers Prev 2020; 29: 539-48.

34. Huangfu SC, Zhang WB, Zhang HR, et al. Clinicopathological and prognostic significance of *Fusobacterium nucleatum* infection in colorectal cancer: a meta-analysis. J Cancer 2021; 12: 1583-91.