Editorial Commentary

The more we learn, the less we know: deciphering the link between human gut fusobacteria and colorectal cancer

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It is becoming increasingly evident that the human gut microbiome is associated with the development of certain diseases. We have recently reviewed the link between specific bacterial taxa and colorectal cancer (CRC) (1). Numerous studies have compared the gut microbial composition of CRC patients with healthy populations, and irrespective of biogeography, the phylum Fusobacteria, the associated genus *Fusobacterium*, and more specifically, the species *Fusobacterium nucleatum* are very frequently found to be enriched in CRC patients, regardless of sample type or detection method (2-7). The role of *F. nucleatum* in CRC is largely attributed to the secretion of virulence factors FadA and Fap2 (8). Cell models have shown the virulence potential of *F. nucleatum* is mediated through FadA and Fap2 by facilitating adherence and invasion, inducing inflammatory and oncogenic pathways, and suppressing immune responses (9,10). To date, *F. nucleatum* is the only *Fusobacterium* taxon strongly associated with CRC, however, other *Fusobacterium* species such as *Fusobacterium periodonticum*, *Fusobacterium varium*, *Fusobacterium ulcerans*, *Fusobacterium necrophorum*, and *Fusobacterium gonidiaformans* have been detected in CRC patients (11). Additionally, non-*nulceatum Fusobacterium* have been associated with other diseases, for example, *F. varium* is associated with ulcerative colitis (12), *F. ulcerans* with tropical ulcers (13) and *F. necrophorum* with Lemierre's syndrome (14). However, the role of non-*nucleatum Fusobacterium* species in CRC, if any, is yet to be elucidated.

In the study by Yeoh *et al.* (15), a shotgun metagenomics approach was used to compare the gut microbial composition of CRC and non-CRC patients (642 CRC *vs.* 2515 non-CRC gut metagenomes) comprising 16 populations from various biogeographies, including southern Chinese and Western cohorts, with a focus on the prevalence and relative abundances of non-*nucleatum Fusobacterium* sp. and the distribution of virulence genes associated with CRC. From an evolutionary and lineage perspective, this approach makes sense: the genus *Fusobacterium* contains non-*nucleatum* species implicated in several pathologies (12-14,16) and pathogenicity may be associated with specific genes or gene repertoires, which through genetic exchange mechanisms or speciation, can be acquired by several species of the same genus (17). Furthermore, it has been suggested based on in silico analyses that horizontal gene transfer may play a role in the evolution of *Fusobacterium* virulence (18). However, while the identification of virulence-related genes using computational tools is often used to infer potential pathogenicity, the establishment of a link with a virulent phenotype does not always follow (19).

There are limited studies on the distribution of *Fusobacterium* species in the gut microbiota of healthy populations. Data from Yeoh *et al.* (15) indicated that several non-nucleatum *Fusobacterium* species are more prevalent (39% *vs.* 7%) and relatively abundant (0.4% *vs.* 0.04%) in southern Chinese metagenomes compared to Western and rural cohorts, irrespective of CRC status. Overall, the microbial composition in stool metagenomes significantly differed between non-CRC populations. Interestingly, they observed differences in the Firmicutes-to-Bacteroidetes ratio between several populations, with Chinese and US populations having higher levels of Bacteroidetes compared

with Firmicutes (63% *vs.* 30%), whereas European populations had higher levels of Firmicutes compared with Bacteroidetes (55% *vs.* 29%). Although, significant differences in the Firmicutes-to-Bacteroidetes ratio are consistently observed between diseased and healthy populations, including CRC (20), whether or not this balance contributes to disease, is yet to be fully determined. Additionally, Yeoh *et al.* (15) reveal differences between non-CRC cohorts, with *Fusobacterium* more relatively abundant in Chinese and Spanish populations (0.47% *vs.* 0.01%). Taking this further, *Fusobacterium mortiferum*, *F. nucleatum*, *F. ulcerans* and *F. varium* were significantly more abundant in the non-CRC metagenomes from Chinese individuals compared to their Western counterparts. While the authors acknowledge biogeography as a possible driving factor for the observed differences in taxonomic profiles between non-CRC cohorts, it is highly likely that diet and other lifestyle factors associated with the different cultures play an important role (21). The authors also compared the relative abundances of *Fusobacterium* species within CRC and non-CRC metagenomes. They detected *F. nucleatum* in all CRC metagenomes, thereby providing further evidence of an association between *F. nucleatum* and CRC. Interestingly, Hong Kong and French populations had higher average relative abundances of *Fusobacterium* taxa than those from the USA, Germany, and Austria. It was also apparent that relative abundances and the prevalence of *F. nucleatum*, *F. gonidiaformans* were higher in six CRC cohorts and *F. peridonticum* and *F. varium* were increased in five of six CRC cohorts compared with non-CRC cohorts. However, only *F. nucleatum* and *F. varium* were significantly associated with CRC. This finding suggests the existence of a genus level CRC complex, with multiple *Fusobacterium* species possibly implicated in the disease, rather than a *F. nucleatum* complex.

Notably, the last update on the taxonomic profile of *Fusobacterium* was in 2002, whereby Citron described 13 characterised species (22). When one considers that there are 366 *Fusobacterium* genome assemblies available in the Refseq NCBI database and 28 *Fusobacterium* species are listed by the List of Prokaryotic names with Standing in Nomenclature (LPSN) at the time of writing this commentary, an updated taxonomic profile based on a comparative genomic analysis of the *Fusobacterium* genus is warranted. Yeoh and colleagues (15) expanded our knowledge on the evolutionary relationship of *Fusobacterium* genomes by including metagenome-assembled genomes (MAGs) from Chinese populations with Western populations and 152 fusobacterial reference genomes downloaded from the Refseq database, in a phylogenetic analysis. Dereplication of 663 genomes resulted in a phylogenetic tree of 218 unique fusobacterial genomes, comprising four monophyletic lineages. Interestingly, the inclusion of MAGs from Chinese cohorts increased the phylogenetic diversity by 14.3% (characterised by branch length), indicating novel diversity within the *Fusobacterium* genus, and more specifically, in the gut microbiota of Chinese individuals. Furthermore, they identified potential new species based on pairwise average nucleotide identity (ANI) comparisons.

The species *F. nucleatum* is now considered a bacterial pathogen implicated in CRC, and, as noted above, one proposed mechanism responsible for carcinogenicity is attributed to the virulence proteins FadA (9) and Fap2 (23). Therefore, is it plausible that related taxa harbouring the same genetic determinants encoding these proteins may also contribute to the development of CRC. Yeoh *et al.* (15) screened fusobacterial MAGs for CRC-associated features and FadA and Fap2 homologues using ≥30% sequence identity and ≥70% alignment length to reference genes as thresholds for homology. They identified 999 FadA homologues in 311 genomes and 754 putative Fap2 homologues in 288 genomes, which included *F. necrophorum*, *F. varium*, and *F. ulcerans*. The identification of FadA homologues in multiple *Fusobacterium* lineages largely coincided with Fap2 homologues, however, FadA was identified in the absence of Fap2 and vice versa, which may also be evidence of genetic exchange events such as recombination. The authors also identify genes in multiple lineages associated with amino acid degradation and iron scavenging, as well as other genes which may be a driver of carcinogenesis. Taken together, the identification of CRC-related genes in multiple *Fusobacterium* lineages, especially of those encoding virulence factors implicated in the disease, gives weight to the suggestion that non-*nucleatum* species may contribute to CRC. The authors state that the prevalence and relative abundance of some species harbouring FadA and Fap2 homologues was increased in CRC populations compared to non-CRC cohorts, an observation that certainly requires further attention.

In summary, Yeoh *et al.* (15) have provided a comprehensive analysis on the relationship between CRC and *Fusobacterium* taxa, especially highlighting the potential involvement of non-*nucleatum Fusobacterium* in the disease. They have contributed to the knowledge of the *Fusobacterium*-CRC association and in doing so

have also raised a number of interesting questions that highlight research gaps on the topic. These gaps are largely a consequence of the lack of data relating non-*nucleatum Fusobacterium* to disease, especially CRC. For instance, they highlight the presence of *Fusobacterium* taxa, including non-*nucleatum* species, in non-CRC southern Chinese populations that are not detected in other cohorts. A logical follow question is: are healthy populations with increased levels of *Fusobacterium* taxa at increased risk of CRC development? Furthermore, if multiple non-*nucleatum Fusobacterium* were enriched in CRC patients, are non-*nucleatum* strains prevalent in other CRC metagenomic datasets? Multiple *Fusobacterium* species were detected in individual CRC populations. Could the presence of multiple *Fusobacterium* species within the gut microbiota be a driver of CRC? Given that novel *Fusobacterium* taxa were identified when compared to reference *Fusobacterium* genomes, is it now time for a comprehensive update to the taxonomy of *Fusobacterium*? Genomic features and homologues of characterised virulence factors FadA and Fap2 were identified in multiple fusobacterial lineages implicating non-nucleatum *Fusobacterium* in CRC. Quantitative polymerase chain reaction (qPCR)-based detection of FadA and Fap2 in non-*nucleatum Fusobacterium* isolates from CRC patients would strengthen this association. And finally, as previous studies on the mucosal (24) and tissue (25) microbiota of CRC patients have also revealed enrichment of *F. nucleatum* compared to non-diseased controls, would similar non-*nucleatum Fusobacterium* trends be observed? There is little doubt that further investigation into the role of non-*nucleatum* taxa harbouring FadA and Fap2 homologues in the development of CRC, and other diseases is needed. Further to this, detailed knowledge of the association between human gut bacteria and the development and progression of CRC may be exploited in CRC prevention strategies, such as the use of antimicrobial-producing probiotics to target CRC-associated bacterial species, which may ultimately decrease the risk of CRC (1).

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References

1. Lawrence GW, Begley M, Cotter PD, et al. Potential Use of Biotherapeutic Bacteria to Target Colorectal Cancer-Associated Taxa. Int J Mol Sci 2020;21:924.
2. Flanagan L, Schmid J, Ebert M, et al. Fusobacterium nucleatum associates with stages of colorectal neoplasia development, colorectal cancer and disease outcome. Eur J Clin Microbiol Infect Dis 2014;33:1381-90.
3. Yamaoka Y, Yutaka Suehiro B, Shinichi Hashimoto B, et al. Fusobacterium nucleatum as a prognostic marker of colorectal cancer in a Japanese population. J Gastroenterol 2018;53:517-24.
4. Mima K, Nishihara R, Qian ZR, et al. Fusobacterium nucleatum in colorectal carcinoma tissue and patient prognosis. Gut 2016;65:1973-80.
5. Tunsjø HS, Gundersen G, Rangnes F, et al. Detection of Fusobacterium nucleatum in stool and colonic tissues from Norwegian colorectal cancer patients. Eur J Clin Microbiol Infect Dis 2019;38:1367-76.
6. Castellarin M, Warren RL, Freeman JD, et al. Fusobacterium nucleatum infection is prevalent in human colorectal carcinoma. Genome Res 2012;22:299-306.
7. Zhou Z, Chen J, Yao H, et al. Fusobacterium and Colorectal Cancer. Front Oncol 2018;8:371.
8. Zhang S, Cai S, Ma Y. Association between Fusobacterium

nucleatum and colorectal cancer: Progress and future directions. J Cancer 2018;9:1652-9.
9. Rubinstein MR, Wang X, Liu W, et al. Fusobacterium nucleatum promotes colorectal carcinogenesis by modulating E-cadherin/β-catenin signaling via its FadA adhesin. Cell Host Microbe 2013;14:195-206.
10. Gur C, Ibrahim Y, Isaacson B, et al. Binding of the Fap2 Protein of Fusobacterium nucleatum to Human Inhibitory Receptor TIGIT Protects Tumors from Immune Cell Attack. Immunity 2015;42:344-55.
11. Hussan H, Clinton SK, Roberts K, et al. Fusobacterium's link to colorectal neoplasia sequenced: A systematic review and future insights. World J Gastroenterol 2017;23:8626-50.
12. Ohkusa T, Sato N, Ogihara T, et al. Fusobacterium varium localized in the colonic mucosa of patients with ulcerative colitis stimulates species-specific antibody. J Gastroenterol Hepatol 2002;17:849-53.
13. Adriaans B, Shah H. Fusobacterium ulcerans sp. nov. from Tropical Ulcers. International Journal of Systematic Bacteriology 1988;38:447-8.
14. Riordan T. Human infection with Fusobacterium necrophorum (Necrobacillosis), with a focus on Lemierre's syndrome. Clin Microbiol Rev 2007;20:622-59.
15. Yeoh YK, Chen Z, Wong MCS, et al. Southern Chinese populations harbour non-nucleatum Fusobacteria possessing homologues of the colorectal cancer-associated FadA virulence factor. Gut 2020. [Epub ahead of print]. doi:10.1136/gutjnl-2019-319635.
16. Lee Y, Eun CS, Lee AR, et al. Fusobacterium Isolates Recovered From Colonic Biopsies of Inflammatory Bowel Disease Patients in Korea. Ann Lab Med 2016;36:387-9.
17. Georgiades K, Raoult D. Defining Pathogenic Bacterial Species in the Genomic Era. Front Microbiol 2011;1:151.
18. Ang MY, Dutta A, Wee WY, et al. Comparative Genome Analysis of Fusobacterium nucleatum. Genome Biol Evol 2016;8:2928-38.
19. Wassenaar TM, Gunzer F. The prediction of virulence based on presence of virulence genes in E. coli may not always be accurate. Gut Pathog 2015;19;7:15.
20. Sinha R, Ahn J, Sampson JN, et al. Fecal Microbiota, Fecal Metabolome, and Colorectal Cancer Interrelations. PLoS One 2016;11:e0152126.
21. Conlon MA, Bird AR. The impact of diet and lifestyle on gut microbiota and human health. Nutrients 2014;7:17-44.
22. Citron DM. Update on the Taxonomy and Clinical Aspects of the Genus Fusobacterium. Clin Infect Dis 2002;35:S22-7.
23. Abed J, Emgård JEM, 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.
24. Mira-Pascual L, Cabrera-Rubio R, Ocon S, et al. Microbial mucosal colonic shifts associated with the development of colorectal cancer reveal the presence of different bacterial and archaeal biomarkers. J Gastroenterol 2015;50:167-79.
25. Mima K, Cao Y, Chan AT, et al. Fusobacterium nucleatum in Colorectal Carcinoma Tissue According to Tumor Location. Clin Transl Gastroenterol 2016;7:e200.

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