Editorial

A tiny but crucial player bridging microbes and colonic carcinogenesis

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Comment on: 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-κB, and Up-regulating Expression of MicroRNA-21. Gastroenterology 2017;152:851-66.e24.

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Colorectal cancer (CRC) is one of the most common malignancies in the world (1). The probability of suffering from CRC is approximately 4–5%, and the risk of developing CRC is associated with characteristics such as age, chronic disease history, and lifestyle (2). CRC is caused by mutations in oncogenes, tumor-suppressor genes, and genes related to DNA repair mechanisms. The underlying pathogenic mechanisms are threefold; namely, chromosomal instability (CIN), microsatellite instability (MSI), and CpG island methylator phenotype (CIMP). In CRC, common mutations, chromosomal changes, and translocations reportedly affect important intracellular signaling pathways [WNT, mitogen activated protein kinase (MAPK)/phosphoinositide 3-kinase (PI3K), transforming growth factor (TGF)-β, and TP53] (2). In addition to genetic mutations, alterations in non-coding RNAs, such as microRNAs, also contribute to carcinogenesis.

The intestinal microbiota is involved in the pathogenesis of intestinal diseases (3). *Fusobacterium nucleatum* is a commensal oral microorganism associated with gingivitis and periodontitis. However, this microbe is also involved in non-oral diseases, such as colorectal and pancreatic cancers (4,5). Metagenomics studies have reported enrichment of *F. nucleatum* in human CRC and adenoma tissues, compared with adjacent tissues (6,7). Subsequent *in vitro* and *in vivo* studies showed that *F. nucleatum* accelerates the progression of CRC, suggesting that it induces tumor growth and enhances tumor survival (5,8-10).

MicroRNAs are a family of small (19–22 nucleotides), non-coding RNAs that post-transcriptionally regulate gene expression. Approximately 30% of human genes are regulated by microRNAs. This regulation has implications for numerous important cellular functions. Given the critical regulatory roles of microRNAs, it is unsurprising that they are associated with carcinogenesis. Many microRNAs, including *microRNA-21*, which mediates cell growth and tumor progression, are upregulated in CRC. Human *microRNA-21* (*hsa-miR-21*) is located on chromosome 17q23-1 overlapping with the *TMEM49* gene, a human homologue of rat vacuole membrane protein-1. *MicroRNA-21* is regulated by its own promoter, which contains binding sites for the transcription factors AP-1 and PU.1 (11). *MicroRNA-21* functions in many cell types as an anti-apoptotic and pro-survival factor, and plays an important role in cancer biology, including in adenoma and CRC (12-15).

The study by Yang *et al.* showed that *F. nucleatum* induces proliferation of colon tumor cells by activating *Toll-like receptor 4* (*TLR4*) and the nuclear factor (NF)-κB pathway, leading to increased levels of *microRNA-21* (16). Using an $Apc^{min/+}$ model, they determined that *F. nucleatum* stimulates the immune response and promotes tumorigenesis due to mutations in the *APC* gene. Several miRNAs, including *microRNA-21*, were upregulated by *F. nucleatum* and *microRNA-21* was shown to be a direct target of *F. nucleatum*. That is, *F. nucleatum* upregulates *microRNA-21*

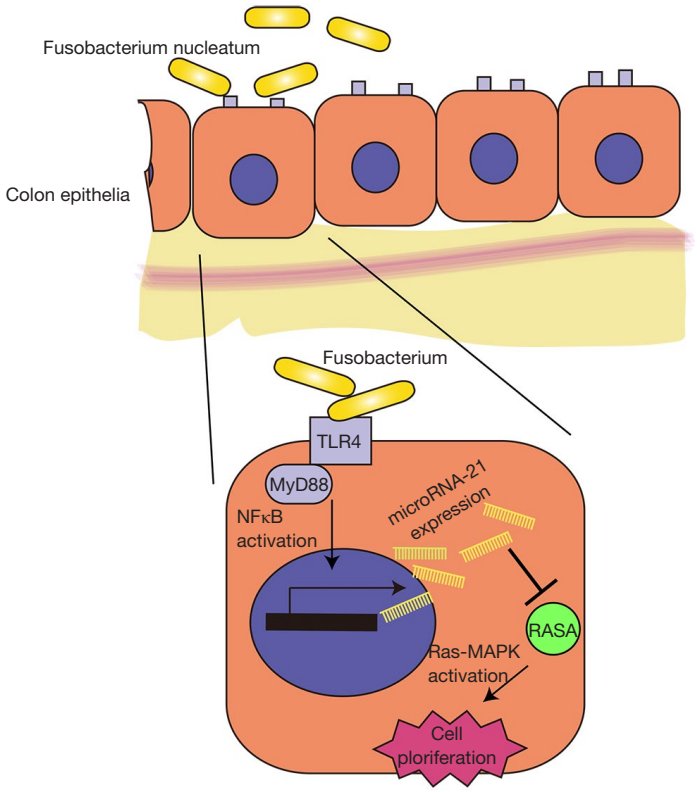

Figure 1 *MicroRNA-21* expression mediates *Fusobacterium nucleatum*-induced proliferation of colon carcinoma cells.

expression by activating *TLR4* and NF-κB. Using *microRNA-21*—knockout (*miR21a*$^{-/-}$) mice, the authors evaluated the role of *microRNA-21* upregulation in colon tumor cell proliferation. In addition, *RASA1*, a suppressor of Ras, was identified as a novel and direct target of *microRNA-21*, which, in turn, activates the MAPK pathway, leading to cell proliferation. These results suggest that *F. nucleatum* plays a role as a driver, not simply a passenger, in colorectal tumorigenesis, by upregulating the expression of *microRNA-21* (*Figure 1*). This study provides solid evidence for an association between *F. nucleatum* and CRC, and suggests that *F. nucleatum* induces colorectal tumorigenesis in a manner dependent on *microRNA-21*.

These novel findings provide interesting insight into the role of *F. nucleatum* in colonic carcinogenesis, a role that involves upregulation of *microRNA-21*. These exciting results shed new light on this field, but also suggest further important questions.

The strain diversity of *F. nucleatum* should be taken into consideration in any future works. Similarly, *F. nucleatum* exists in a complex community of other microorganisms. Thus, it may be necessary to investigate the role of the intestinal microbiota in *F. nucleatum*-induced colonic carcinogenesis. Such analyses may provide information on why *F. nucleatum* induces CRC at a specific stage of life or in specific cases. In addition, while this may also be related to strain diversity, the *F. nucleatum* virulence factors involved in colonic carcinogenesis should be determined. Fusobacterial lectin Fap2 reportedly interacts with host Gal-GalNac (17), and fusobacterial FadA adheres to host cadherins, which leads to activation of oncogenic beta-catenin signaling (10). These results, together with the findings of Yang and colleagues (16), suggest that *F. nucleatum* interferes with host cellular homeostasis via multiple pathways.

Another important question regarding *F. nucleatum*-related colonic carcinogenesis is the global prevalence of this agent. There are significant differences in the prevalence of *F. nucleatum* in CRCs among countries. For example, 13% of colon carcinoma tissues were *F. nucleatum* positive in a study in the United States (4), while only 8.6% were positive in Japanese cases (4). Thus, the causative role

of this pathogen in colonic carcinogenesis should take into consideration the geographical differences. Other microbes may also play crucial roles in colonic carcinogenesis in some countries. Thus, the significance in colonic carcinogenesis and distribution of *F. nucleatum* should be clarified further. In addition, because *microRNA-21* is overexpressed in most solid tumors, it is possible that microbes other than *F. nucleatum* induce *microRNA-21* expression.

MicroRNA-21 is commonly overexpressed in solid tumors, such as those of the lung, breast, stomach, prostate, colon, brain, head and neck, esophagus, and pancreas (15). *MicroRNA-21* reportedly targets *PDCD4*, *CdC25A*, *TGF-β R2*, or other genes, some of which may be involved in colonic carcinogenesis. The study by Yang *et al.* suggested *RASA* as a novel target of *microRNA-21*, which promotes CRC cell proliferation. However, *RASA* is likely not the only gene responsible for the induction of *microRNA-21*-mediated colonic carcinogenesis.

Regulation of microRNA expression is a hot topic. *c-fos* and the *AP-1* family are involved in *miroRNA-21* promoter activity (11). Yang *et al.* found that NF-κB influenced *microRNA-21* promoter activity, which is consistent with a previous report (18). However, *microRNA-21* also targets Pelino, which negatively regulates NF-κB activity (19). Thus, the mechanism by which *F. nucleatum* activates the *microRNA-21* promoter, and whether this is provoked solely by activation of the NF-κB pathway, should be subjects of future works.

CRCs can be classified as sporadic, inflammation-associated, or inherited. The contribution of *F. nucleatum* to colorectal carcinogenesis in general is unclear. For example, colitis-associated cancer may be different pathologically from sporadic or inherited cancers. Indeed, although microRNA expression levels are unchanged, microRNA function is globally deregulated in the presence of chronic inflammation (20), which is closely associated with colitis-associated carcinogenesis (21). Because Yang *et al.* used dextran sulfate sodium (DSS)-induced colitis-associated colon cancer model to evaluate the role of *F. nucleatum*, it will be interesting to assess microRNA function in *F. nucleatum*-infected lesions.

A crucial question regarding *F. nucleatum* and colonic carcinogenesis is whether this pathogen induces the genetic abnormalities detected in most CRCs. There is some speculation about an indirect linkage between *F. nucleatum* and MSI due to reduced activity of mismatch repair mechanisms caused by reactive oxygen species (ROS) produced as a result of infection (8). However, no direct linkage between *F. nucleatum* infection and induction of genetic mutations has been reported to date. Yang *et al.* used *in vivo* $Apc^{min/+}$ and azoxymethane (AOM)/DSS models, which may involve genetic mutations in *Apc* or random mutations caused by AOM in the absence of *F. nucleatum* infection. Then, strictly speaking, *F. nucleatum* may not be a pure driver of carcinogenesis, but accelerates the proliferation of colon cancer cells by inducing *microRNA-21* expression. Therefore, a relationship between the *F. nucleatum* level and/or *microRNA-21* expression in carcinoma tissues and prognosis is feasible, and these may be clinically useful biomarkers.

As described above, many issues remain to be determined to gain a full understanding of the biological role of *F. nucleatum* in colon cancers. However, the findings of Yang *et al.* will facilitate the development of novel therapeutics. Whether eradication of *F. ucleatum* or inhibition of *microRNA-21* function improves the prognosis of a subset of colon cancer patients should be determined. Also, the involvement of microRNAs in the roles of pathogens in pathological conditions should be investigated not only in colon cancers but also in other diseases. It is possible that, similar to *F. nucleatum*, as-yet-unknown agents regulate microRNA expression in pathological conditions. Efforts to discover such factors will contribute to improvement of human health. In this context, the association between microbes and microRNAs in human diseases is a hot topic that warrants vigorous research.

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