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Fusobacterium nucleatum and colorectal cancer: A review

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Dear Editors and Reviewers：

We thank the reviewers very much for these comments. These suggestions are definitely very helpful for us to make this manuscript better. Thank you again.

Reviewer # 1 (Comments to the Author):

The review article by Shang and Liu entitled "*Fusobacterium nucleatum* and Colorectal Cancer: a review" is an overview on recent literature on the possible role of Fusobacterium nucleatum in colorectal carcinogenesis. Reported studies show that there is still controversy on the importance of that bacterial strain in affecting colonic tumors, though the mechanisms proposed to explain those effects are largely convincing, especially those involving the inflammatory and immune environment of normal and tumor cells in the large bowel. However, the overall impression is that it is a long way to walk… The paper is fairly well written, though some English polishing is needed.[i.e, the title of paragraph "F. Bacterium and Immunity Status (and not Statue) in Colorectal Cancer" on page 10, or some verbs not in line with the subject(s), fecal instead of feces on page 6, paragraph 2, line 4, and so on…]

Response: Thank you for your comments. Our manuscripts have been edited by the highly qualified native English speaking editors. Some of the wrong words that appeared the manuscript have been corrected, and the language has been polished. Thank you very much!

Reviewer # 2 (Comments to the Author):

M1: Inaccurate statement

Response: Thank you for your guidance. The original statement was：

It also has been documented to be involved in pancreatic cancer, oral cancer, premature and term stillbirths [27-30].

Now it is：

It has also been associated with pancreatic cancer, oral cancer, and premature and term stillbirths [27-30].

M2: What are the purported mechanisms and their relation to CRC?

Response: *F. nucleatum* is closely connected with liver abscess, appendicitis and infections of the head and neck, including mastoiditis, tonsillitis and maxillary sinusitis. Many studies have demonstrated the importance and synergistic potentials of *Fusobacterium spp.* relative to other organisms in mixed infections, while the specific mechanism is not clear. *F. nucleatum* has the ability to trigger the colonic epithelium cell damage directly, consequently contributing to the development of CRC.

M3: What are the bases for this allegation? Please, clarify.

Response: The amount of tissue *F. nucleatum* DNA is associated with higher colorectal cancer-specific mortality, independent of clinical, pathological and major tumour molecular features, which shows that a higher abundance of *F. nucleatum* in CRC is associated with a shorter survival time.

M4: Please, clarify!

Response: The researchers have found that the *Fusobacterium*-high colorectal cancer group is associated with *CIMP* positivity (p=0.001), *TP53* wild-type (P=0.015), *MSI* (P=0.018), and *CHD7/8* mutation positivity (p=0.002)[45].

Mima K, et al. have found that a higher amount of tissue *F. nucleatum* DNA is associated with *CIMP*-high and *BRAF* mutation (p≤0.001)[44].

M6: What is the relation between this factor and/ or the effect of this factor on the purported association/ causation?

M7: What are the reasons for this opinion? Please, clarify.

M6 and M7 Response: *F. nucleatum* was previously regarded as a passenger

bacterium in human intestinal tract[46,47]. while it has been confirmed to be associated with specific tumor molecular events, including CpG island methylator phenotype (CIMP), microsatellite instability (MSI), and genetic mutations in *BRAF*, *CHD7, CHD8* and *TP53*[44,45], recently. Taken together, these findings indicate that *Fusobacterium* enrichment is associated with specific molecular subsets of CRC, offering support for a pathogenic role in CRC for this gut microbiome component instead of a passenger bacterium in human intestinal tract.

M8: What is the mechanism?

Response: Some investigators utilized mice that develop intestinal tumors because of a mutation in one copy of the tumor suppressor gene *Apc* or genetic defects that lead to chronic intestinal inflammation. Introduction of *F. nucleatum* into $Apc^{Min/+}$ mice accelerated the onset of colonic tumors. These researchers found that $Apc^{Min/+}$ mice fed *F. nucleatum* developed a significantly higher number of colonic tumors compared with control groups, which results in colitis and promotes colorectal tumorigenesis in $Apc^{min/+}$ mice.

M9: The authors must put in writing clear purported pathogenesis!

Response: The original statement was：

Rubinstein *et al*[43] have reported that *F. nucleatum* stimulates tumor cell growth in CRC by activating β-catenin signaling *via* FadA adhesion.

Now it is：

Rubinstein *et al*[43] have reported that *F. nucleatum* stimulates tumor cell growth in CRC by activating β-catenin signaling and inducing oncogenic gene expression *via* the FadA adhesion virulence factor.

M10: Please, clarify!

Response: The original statement was：

Together, these studies support that *F. nucleatum* is a cause of CRC rather than

a consequence.

Now it is：

Together, these studies show that *F. nucleatum* plays an important role in the initiation of CRC and promoting tumor cell growth in CRC, supporting that *F. nucleatum* is a cause of CRC rather than a consequence.

M11: This supports the idea of association rather than causation!

Response: The original statement was：

This study suggests that positive rates of *F. nucleatum* may be also different according to intestinal sites.

Now it is：

The percentage of *F. nucleatum*-enriched CRC gradually increases from rectum to cecum[51], suggesting that the rate at which *F. nucleatum* is present may also differ among intestinal sites.

M12: Poor English language.

M13: Lack of coherent pathogenesis!

M12 and M13 Response: Thank you for your comments. The language has been polished, recently. Underlying mechanisms of *F. nucleatum* pathogenesis in colorectal cancer have been shown in Figure 1, clearly. And our manuscripts have been edited by the highly qualified native English speaking editors. Thank you again!