

## PEER-REVIEW REPORT

**Name of journal:** *World Journal of Gastroenterology*

**Manuscript NO:** 73159

**Title:** Salivary Fusobacterium nucleatum serves as a potential diagnostic biomarker for gastric cancer

**Provenance and peer review:** Unsolicited manuscript; Externally peer reviewed

**Peer-review model:** Single blind

**Reviewer's code:** 03252941

**Position:** Editorial Board

**Academic degree:** MD

**Professional title:** Doctor, Professor

**Reviewer's Country/Territory:** Japan

**Author's Country/Territory:** China

**Manuscript submission date:** 2021-11-26

**Reviewer chosen by:** AI Technique

**Reviewer accepted review:** 2021-11-28 08:33

**Reviewer performed review:** 2021-12-06 09:01

**Review time:** 8 Days

Scientific quality	<input type="checkbox"/> Grade A: Excellent <input checked="" type="checkbox"/> Grade B: Very good <input type="checkbox"/> Grade C: Good <input type="checkbox"/> Grade D: Fair <input type="checkbox"/> Grade E: Do not publish
Language quality	<input type="checkbox"/> Grade A: Priority publishing <input checked="" type="checkbox"/> Grade B: Minor language polishing <input type="checkbox"/> Grade C: A great deal of language polishing <input type="checkbox"/> Grade D: Rejection
Conclusion	<input type="checkbox"/> Accept (High priority) <input type="checkbox"/> Accept (General priority) <input checked="" type="checkbox"/> Minor revision <input type="checkbox"/> Major revision <input type="checkbox"/> Rejection
Re-review	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No

<b>Peer-reviewer statements</b>	Peer-Review: [ <input checked="" type="checkbox"/> ] Anonymous [ <input type="checkbox"/> ] Onymous
	Conflicts-of-Interest: [ <input type="checkbox"/> ] Yes [ <input checked="" type="checkbox"/> ] No

## SPECIFIC COMMENTS TO AUTHORS

In this study, Chen WD et al. reported close associations of salivary fusobacterium nucleatum (Fn) with gastric cancer (GC) and its progression. In addition, the demonstrated that Fn enhances motility and invasiveness of GC cells and is also implicated in epithelial-mesenchymal transition of GC cells. This study is well designed, methodology is appropriate, results are beautiful, and discussion is valid. I think that this manuscript is worth publishing. To further improve this manuscript, I have some suggestions. 1. (p.5, ll.10-12) Most patients have peritoneal metastasis or liver metastasis when diagnosed: I think that this description is out-of-date and that fewer GC would be found in such an advanced stage today. Please search the literature well. 2. (p.10. l.16) median and range: Is this range SD, SEM, or interquartile range? Please specify. 3. (p.16, Figure 4) What is the difference between migration and invasion investigated by Transwell assay? If migration assay is different from invasion, please describe the experimental procedure in the corresponding section. 4. (p.19, ll.5-12): My largest concern is whether or not H. pylori may be a confounding factor between GC and Fn infection. Poor hygiene status would be associated with both Fn and H. pylori infection. In fact, Fn DNA levels were high not only in GC but in atrophic gastritis that is closely associated with H. pylori infection. Meanwhile, H. pylori may become underpresented in completed atrophic gastritis, which is an origin of intestinal-type GC. How about the possibility that H. pylori hidden behind Fu is actually the cause of GC?

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**Peer-review model:** Single blind

**Reviewer's code:** 05222666

**Position:** Peer Reviewer

**Academic degree:** MD

**Professional title:** Doctor

**Reviewer's Country/Territory:** Italy

**Author's Country/Territory:** China

**Manuscript submission date:** 2021-11-26

**Reviewer chosen by:** AI Technique

**Reviewer accepted review:** 2021-11-26 14:41

**Reviewer performed review:** 2021-12-13 02:14

**Review time:** 16 Days and 11 Hours

<b>Scientific quality</b>	<input type="checkbox"/> Grade A: Excellent <input type="checkbox"/> Grade B: Very good <input checked="" type="checkbox"/> Grade C: Good <input type="checkbox"/> Grade D: Fair <input type="checkbox"/> Grade E: Do not publish
<b>Language quality</b>	<input type="checkbox"/> Grade A: Priority publishing <input checked="" type="checkbox"/> Grade B: Minor language polishing <input type="checkbox"/> Grade C: A great deal of language polishing <input type="checkbox"/> Grade D: Rejection
<b>Conclusion</b>	<input type="checkbox"/> Accept (High priority) <input type="checkbox"/> Accept (General priority) <input type="checkbox"/> Minor revision <input checked="" type="checkbox"/> Major revision <input type="checkbox"/> Rejection
<b>Re-review</b>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No

<b>Peer-reviewer statements</b>	Peer-Review: [ <input checked="" type="checkbox"/> ] Anonymous [ <input type="checkbox"/> ] Onymous Conflicts-of-Interest: [ <input type="checkbox"/> ] Yes [ <input checked="" type="checkbox"/> ] No
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## SPECIFIC COMMENTS TO AUTHORS

The manuscript entitled “Salivary *Fusobacterium nucleatum* serves as a potential biomarker and plays malignant biological role in gastric cancer” by Wen-Dan Chen, Xin Zhang, Meng-Jiao Zhang, Ya-Ping Zhang, Zi-Qi Shang, Yi-Wei Xin, Yi Zhang, presents evidence that Fn abundance in saliva could be used as a promising biomarker to diagnose gastric cancer (GC). Moreover, the Authors suggest that Fn infection could promote GC metastasis by accelerating the EMT process. In particular, the Authors demonstrated Fn abundance in saliva by digital droplet polymerase chain reaction (ddPCR), and established a new simple and effective diagnostic approach to improve the early diagnosis rate of GC. • ddPCR results showed that among the patients with GC and benign gastric disease, and HCs, the Fn level was significantly higher in GC patients compared with atrophic gastritis (AG), non-atrophic gastritis (NAG), gastric polyps (GP) patients and healthy controls (HCs) while there was no difference among AG, NAG, GP patients and HCs; • Moreover, the Fn level was increased with the TNM stage; the Fn level in GC patients with lymph node metastasis was significantly higher compared with those without lymph node metastasis ( $p < 0.001$ ); • The Fn level was significantly higher in GC patients compared with AG, NAG, GP patients and HCs, while CEA, CA199, CA724 and ferritin did not significantly different between GC patients and the other four groups. • The effects of Fn infection in vitro was investigated in infected BGC823 and SGC7901 cells with Fn by the transwell assay and wound-healing assay in the absence or presence of the infected cells. The results obtained in transwell assay indicated that Fn infection significantly enhanced the invasive and migratory capacities of BGC823 and SGC7901 cells; • These results were confirmed by the wound-healing assay. Since EMT

is an important process of metastasis, the Authors examined the impact of Fn infection on the expressions of proteins involved in the EMT process by Western blotting analysis. The results revealed that Fn infection significantly decreased the expressions of epithelial markers, such as E-cadherin, while it increased the expressions of mesenchymal phenotype-associated molecules, such as N-cadherin, vimentin and Snail. This is a novel topic that will be of interest to the readers of the journal. Moreover, conclusions are supported by an appropriate number of evidence. However, I personally consider the paper acceptable upon revisions since some points need to be addressed before the results are published. Specific comments: 1) The Authors should provide evidence (for example through STR DNA profiling ) that the two cell lines - BGC823 and SGC7901 - were used in the in vitro experiments are derived from gastric adenocarcinoma. 2) The Authors should describe in the Introduction the role of *F. nucleatum* in determining an imbalance in the commensal bacterial composition of oral cavity. In fact, it is known that Fn is among the pathobionts that outgrow during dysbiosis preceding periodontal disease (see Nozawa et al., 2020). 3) The Authors should explain why they used an MOI of 100 in the transwell and wound healing assays. Minor comments THE CONTENT OF SOME SECTIONS MUST BE IMPROVED Multiple parts of this manuscript are poorly written. Word vocabulary and grammar need to be improved. Some examples: -page 3, Abstract section, Background “As one of the most common tumors, gastric cancer (GC) has a high mortality rate. Since current examination approaches cannot achieve early diagnosis.” CHANGE as follows: “As one of the most common tumors, gastric cancer (GC) has a high mortality rate, since current examination approaches cannot achieve early diagnosis”; -Write *Fusobacterium nucleatum* as well as *Helicobacter pylori* in Italics throughout the text: i.e. *Fusobacterium nucleatum*. -page 4, Results section “Importantly, the Western blotting analysis further presented that Fn infection significantly decreased the expression of E-cadherin and increased the expressions of



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N-cadherin, vimentin and Snail.” CHANGE TO “Importantly, the Western blotting analysis further showed that Fn infection significantly decreased the expression of E-cadherin and increased the expressions of N-cadherin, vimentin and Snail.”. -page 4, Core tip “Further cell experiments revealed that Fn could promote the migration and invasion of GC cells by promoting the EMT process” CHANGE TO “Further, experiments in vitro revealed that Fn could promote the migration and invasion of GC cells by promoting the EMT process”. -page 6, a reference to the sentence “Fusobacterium nucleatum (Fn) is a Gram-negative anaerobic bacterium, which is essential for the normal oral microenvironment” is lacking. Please, add a reference consistently. -page 7: CHANGE Methods and Meterials TO Methods and Materials. -page 7: please add the code number of Ethics Committee approval. -page 8: complete the sentence “Briefly, after an initial enzyme activation step at 50°C for 10 min and then at 90°C for 10 min, the amplification .....????? were carried out”. -page 9: CHANGE “the cells suspension” TO “the cell suspensions”. -page 13: CHANGE CA199 to CA19-9 and CA724 TO CA72-4. -page 17: add legend to the figure of Western blot assay and CHANGE E-cadhein TO E cadherin. -page 20: please, cut the sentence “EMT is a special program that enables settled epithelial cells to gain the ability to migrate as single cells, which can enhance mobility, invasion, and resistance to apoptosis, conferring metastatic properties of cancer cells[26].” and paste it after the sentence “EMT is a classical pathway promoting metastasis.”.

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**Peer-review model:** Single blind

**Reviewer's code:** 06045450

**Position:** Peer Reviewer

**Academic degree:** MD

**Professional title:** Assistant Professor

**Reviewer's Country/Territory:** Iran

**Author's Country/Territory:** China

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**Review time:** 8 Days and 11 Hours

Scientific quality	<input type="checkbox"/> Grade A: Excellent <input checked="" type="checkbox"/> Grade B: Very good <input type="checkbox"/> Grade C: Good <input type="checkbox"/> Grade D: Fair <input type="checkbox"/> Grade E: Do not publish
Language quality	<input type="checkbox"/> Grade A: Priority publishing <input checked="" type="checkbox"/> Grade B: Minor language polishing <input type="checkbox"/> Grade C: A great deal of language polishing <input type="checkbox"/> Grade D: Rejection
Conclusion	<input type="checkbox"/> Accept (High priority) <input checked="" type="checkbox"/> Accept (General priority) <input type="checkbox"/> Minor revision <input type="checkbox"/> Major revision <input type="checkbox"/> Rejection
Re-review	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No



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<b>Peer-reviewer statements</b>	Peer-Review: [ <input checked="" type="checkbox"/> ] Anonymous [ <input type="checkbox"/> ] Onymous Conflicts-of-Interest: [ <input type="checkbox"/> ] Yes [ <input checked="" type="checkbox"/> ] No
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#### **SPECIFIC COMMENTS TO AUTHORS**

It is a good study, but it needs to be studied with more patients for a definite conclusion



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**Peer-review model:** Single blind

**Reviewer's code:** 03822338

**Position:** Editorial Board

**Academic degree:** FACS, MBBS, MNAMS, MS

**Professional title:** Professor

**Reviewer's Country/Territory:** India

**Author's Country/Territory:** China

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**Reviewer performed review:** 2021-12-30 20:46

**Review time:** 13 Days and 2 Hours

Scientific quality	<input type="checkbox"/> Grade A: Excellent <input checked="" type="checkbox"/> Grade B: Very good <input type="checkbox"/> Grade C: Good <input type="checkbox"/> Grade D: Fair <input type="checkbox"/> Grade E: Do not publish
Language quality	<input type="checkbox"/> Grade A: Priority publishing <input checked="" type="checkbox"/> Grade B: Minor language polishing <input type="checkbox"/> Grade C: A great deal of language polishing <input type="checkbox"/> Grade D: Rejection
Conclusion	<input checked="" type="checkbox"/> Accept (High priority) <input type="checkbox"/> Accept (General priority) <input type="checkbox"/> Minor revision <input type="checkbox"/> Major revision <input type="checkbox"/> Rejection
Re-review	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No

<b>Peer-reviewer statements</b>	Peer-Review: [ <input checked="" type="checkbox"/> ] Anonymous [ <input type="checkbox"/> ] Onymous Conflicts-of-Interest: [ <input type="checkbox"/> ] Yes [ <input checked="" type="checkbox"/> ] No
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## SPECIFIC COMMENTS TO AUTHORS

Dear Authors, It was a pleasure going through the manuscript. Its is well designed and well scripted. However there are few issues which I am commented alongside the article attached below. Structured comments: 1. Title. Does the title reflect the main subject/hypothesis of the manuscript? YES (But requires some changes s suggested) 2 Abstract. Does the abstract summarize and reflect the work described in the manuscript? YES (Requires some editing as has been suggested) 3 Key words. Do the key words reflect the focus of the manuscript? YES 4 Background. Does the manuscript adequately describe the background, present status and significance of the study? YES 5 Methods. Does the manuscript describe methods (e.g., experiments, data analysis, surveys, and clinical trials, etc.) in adequate detail? YES 6 Results. Are the research objectives achieved by the experiments used in this study? YES What are the contributions that the study has made for research progress in this field? SEARCHING A BIOMARKER FOR POSSIBLE DETECTION OF CANCER STOMACH 7 Discussion. Does the manuscript interpret the findings adequately and appropriately, highlighting the key points concisely, clearly and logically? YES Are the findings and their applicability/relevance to the literature stated in a clear and definite manner?YES Is the discussion accurate and does it discuss the paper's scientific significance and/or relevance to clinical practice sufficiently? YES 8 Illustrations and tables. Are the figures, diagrams and tables sufficient, good quality and appropriately illustrative of the paper contents? YES Do figures require labeling with arrows, asterisks etc., better legends? 9 Biostatistics. Does the manuscript meet the requirements of biostatistics? YES 10 Units. Does the manuscript meet the requirements of use of SI units? YES 11 References. Does



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the manuscript cite appropriately the latest, important and authoritative references in the introduction and discussion sections? Does the author self-cite, omit, incorrectly cite and/or over-cite references? YES 12 Quality of manuscript organization and presentation. Is the manuscript well, concisely and coherently organized and presented? YES Is the style, language and grammar accurate and appropriate? NO 13 Research methods and reporting. Authors should have prepared their manuscripts according to manuscript type and the appropriate categories, as follows: (1) CARE Checklist (2013) - Case report; (2) CONSORT 2010 Statement - Clinical Trials study, Prospective study, Randomized Controlled trial, Randomized Clinical trial; (3) PRISMA 2009 Checklist - Evidence-Based Medicine, Systematic review, Meta-Analysis; (4) STROBE Statement - Case Control study, Observational study, Retrospective Cohort study; and (5) The ARRIVE Guidelines - Basic study. Did the author prepare the manuscript according to the appropriate research methods and reporting? 14 Ethics statements. For all manuscripts involving human studies and/or animal experiments, author(s) must submit the related formal ethics documents that were reviewed and approved by their local ethical review committee. Did the manuscript meet the requirements of ethics? OK

## RE-REVIEW REPORT OF REVISED MANUSCRIPT

**Name of journal:** *World Journal of Gastroenterology*

**Manuscript NO:** 73159

**Title:** Salivary Fusobacterium nucleatum serves as a potential diagnostic biomarker for gastric cancer

**Provenance and peer review:** Unsolicited manuscript; Externally peer reviewed

**Peer-review model:** Single blind

**Reviewer's code:** 05222666

**Position:** Peer Reviewer

**Academic degree:** MD

**Professional title:** Doctor

**Reviewer's Country/Territory:** Italy

**Author's Country/Territory:** China

**Manuscript submission date:** 2021-11-26

**Reviewer chosen by:** Jing-Jie Wang

**Reviewer accepted review:** 2022-02-03 09:14

**Reviewer performed review:** 2022-02-03 12:30

**Review time:** 3 Hours

<b>Scientific quality</b>	<input type="checkbox"/> Grade A: Excellent <input checked="" type="checkbox"/> Grade B: Very good <input type="checkbox"/> Grade C: Good <input type="checkbox"/> Grade D: Fair <input type="checkbox"/> Grade E: Do not publish
<b>Language quality</b>	<input type="checkbox"/> Grade A: Priority publishing <input checked="" type="checkbox"/> Grade B: Minor language polishing <input type="checkbox"/> Grade C: A great deal of language polishing <input type="checkbox"/> Grade D: Rejection
<b>Conclusion</b>	<input type="checkbox"/> Accept (High priority) <input checked="" type="checkbox"/> Accept (General priority) <input type="checkbox"/> Minor revision <input type="checkbox"/> Major revision <input type="checkbox"/> Rejection
<b>Peer-reviewer</b>	Peer-Review: <input checked="" type="checkbox"/> Anonymous <input type="checkbox"/> Onymous

**statements**

Conflicts-of-Interest: [ ] Yes [Y] No

**SPECIFIC COMMENTS TO AUTHORS**

Manuscript # Number ID: 05222666, entitled "Salivary Fusobacterium nucleatum serves as a potential biomarker and plays malignant biological role in gastric cancer" by Wen-Dan Chen, Xin Zhang, Meng-Jiao Zhang, Ya-Ping Zhang, Zi-Qi Shang, Yi-Wei Xin, Yi Zhang (revised). I appreciated the efforts of the Authors in revising this manuscript. The manuscript has been improved as compared to the previous version. However, I have some more concerns as detailed in the attached file "review of the revised MS 3 February 2022".