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Re: Manuscript NO.: 46385 April 1, 2019
To: World Journal of Gastrointestinal Oncology Editorial office

Dear Dr. Wang,
Thank you for your recent correspondence regarding our manuscript 46385 entitled "**SFRP4 Expression Correlates with EMT-linked Genes and Poor Overall Survival in Colon Cancer Patients**" by Nfonsam et al. We have carefully considered the reviews made and here we are submitting a revised manuscript that addresses point-by-point all the comments of reviewers. All suggested changes are included.
Please find below, a point-by-point description of our responses to the reviewer's comments. For the convenience of the reviewers, any significant changes in the text were underlined.

**Responses to the comments from Reviewer #1**
SFRP4 is a gene that is often suppressed by methylation, and colon cancer cells are also known to exhibit such epigenetic changes. Nevertheless, this paper claims that the expression of SFRP4 is associated with a poor prognosis of patients with colon cancer. This paper also shows that SFRP4 is more expressed in tumors with high tumor stroma ratios and that there exist positive correlations between expression of SFRP4 and EMT-related genes, which is unlikely considering the biological role of SFRP4, inhibition of WNT signaling. Thus, it needs to be clarified whether the expression of SFRP4 occurs in colon tumor cells or in stromal cells. Therefore, the authors should perform experiments exploring whether expression of SFRP4 occurs in stroma and tumor cells suppress the expression of SFRP4 by methylation. This paper is not a treatise on EOCC. The conclusion of the discussion is that EOCC's SFRP4 is a determinant of prognosis. This paper deals with colon cancer from young and old patients, not young patients (EOCC).

**Response:** We thank the reviewer for the insightful comments. We agree with the reviewer that the role of SFRPs including SFRP4 as WNT signaling inhibitors is well documented in the literature. *However, as we mention in the paper (discussion), there is also accumulating evidence suggesting that the SFRP family proteins might also activate WNT signaling and promote cell proliferation, therefore demonstrating oncogenic potential and suggesting that the mechanism of action of SFRPs might be context dependent.*

a) The most understood mechanism of interaction and the mechanism through which SFRPs inhibit WNT signaling is by SFRPs binding and sequestering the Wnt ligand (Finch et al., 1997; Leyns et al., 1997; Wang et al., 1997; Rattner et al., 1997; Lin et al. 1997) due to the sequence similarity of the SFRP N-terminal Cystein-rich domain to the Frizzled receptors. However, there are other types of Wnt interactions that have been reported, all of which suggest activation of WNT signaling:

    1. By binding and sequestering the Wnt ligand, SFRPs may also act as carriers for Wnt proteins in the extracellular space, hence expanding the range and activity of WNT signaling. This has been reported in Xenopus where SFRP3 and Crescent (SFRP family member) were shown to

potentially activate canonical Wnt signaling by promoting the diffusion of Wnt8 and Wnt11 (Mii and Taira 2009).

2. There have also been reports of direct interaction and dimerization of the N-terminal Cystein-rich domain of SFRPs and FZ proteins, hence suggesting SFRP potential activation of WNT signaling by mimicking the Wnt ligand. Dann et al. (2001) revealed a crystal structure of the dimerization of mouse FZ8 and SFRP3, and other groups have reported on the formation of homomeric and heteromeric complexes of SFRPs and FZ (Bafico 1999; Rodriguez et al. 2005).
3. In addition, SFRP2 was shown to enhance WNT3A signaling in HEK293 and HSG cells (von Marschall and Fisher 2010) while in the mouse, SFRP2 activated β-catenin signaling through direct interaction with Fz4/7 (Kress et al. 2009).
4. The SFRPs can also activate WNT signaling by antagonizing each other, such as in rat renal organogenesis where SFRP2 was reported to inhibit SFRP1 activities (Yoshino et al. 2001).
5. Also, WNT signaling regulation could be concentration dependent with low SFRP concentrations activating WNT signaling, and at high concentrations inhibiting it, as shown in Bhat et al (2007).
6. Consistent with concept of SFRPS functioning as oncogenes, SFRP1 has been reported overexpressed in basal-like breast cancers and implicated in brain-specific metastases, while SFRP2 induces mammary tumor formation in canines by interacting with the extracellular matrix and blocking apoptosis (Smid et al. 2008; Lee et al. 2004a; Lee et al. 2004b; Lee et al. 2006b).
7. Both sFRP1 and sFRP2 promote cellular invasion/proliferation and tumor growth in renal cancer, (Saini et al. 2009; Yamamura et al. 2010). A similar observation has also been documented for high SFRP1 expression in gastric cancer.
8. *More interestingly and consistent with our result, overexpression of SFRP4 has also been seen in stomach cancer where it associate with poor patient outcomes, and found to also strongly associate with EMT genes in gliomas (Vincent and Postovit, 2017).*

Taken together, these data suggest that besides being a WNT signaling inhibitor, the SFRP family members also have oncogenic potentials and the mechanism of action of SFRPs is context dependent. We have expanded on this in the discussion section of the manuscript (paragraph 3 and 4).

b) Regarding the tissue source of SFRP, the data reported in this study was not generated by our laboratory. We had previously reported on the overexpression of SFRP4 in early-onset colon cancer using in-house primary samples (Jandova et al., 2016). However, all analysis in the present study was performed using the colon adenocarcinoma (COAD) expression dataset that is available online in the cancer genome atlas (TCGA) website. We agree with the reviewer that it is a possibility that SFRP4 overexpression may have originated from the stroma as documented by the strong correlation between SFPR4 and the stroma in Vincent and Postovit, (2017). We have added this possibility in the discussion section (paragraph 7) of the manuscript. Nevertheless, whether SFRP4 is expressed by the tumor cells and/or the stroma may not be very relevant or beyond the scope of this paper given that the stroma constitutes an integral part of the tumor microenvironment and will always play a vital role in tumor growth and proliferation.

c) The conclusion that early-onset colon cancer expression of SFRP4 is a determinant of prognosis has been expanded upon to add more clarity and address the reviewers concern (Discussion: last paragraph). This conclusion draws from 1) our previous report where we showed that SFRP4 was overexpressed in early-onset colon cancer (Jandova et al., 2016) and 2) our current data which

shows that SFRP4 overexpression correlates with that of EMT genes and associates with poor survival.

**Responses to the comments from Reviewer #2**

The topic and the concept that SFRP4 expression correlates with EMT-linked genes and poor survival in colon cancer are interesting.

***1. In the title EMT should be fully spelled out.***

**Response:** As suggested by the reviewer, EMT was spelled out in the title.

***2. Please provide for the readers more information regarding the Cancer Genome Atlas (TCGA), e.g, who developed it, and data set from TCGS, the authors used, e.g. number of patients in groups.***

**Response:** We would like to thank the review for this insightful comment. We have added a paragraph (see below) describing TCGA data portal in more details to the methodology section.

"A landmark cancer genomics program molecularly characterized over 20,000 primary cancer and matched normal samples spanning 33 cancer types. TCGA was formed in 2005 when the U.S. National Cancer and National Human Genome Research Institutes teamed together to support the launch of the project to comprehensively map various cancer genomic changes (https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga/history/timeline). This joint effort brought together researchers from diverse disciplines and multiple institutions. Over the next dozen years, TCGA generated over 2.5 petabytes of genomic, epigenomic, transcriptomic, and proteomic data. The data, which has already lead to improvements in our ability to diagnose, treat, and prevent cancer, will remain publicly available for anyone in the research community to use (https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga). As of march 2019, the TCGA data portal has contains 365,463 genomic and clinical data for 45 projects; 68 primary sites; 33,549 cases; 22,872 genes and 3,142,246 mutations. The TCGA-COAD (Colon adenocarcinoma) project contains 461 cases including two primary sites, colon and rectosigmoid junction, and following disease types: adenomas and adenocarcinomas, complex epithelial neoplasms; cystic, mucinous and serous neoplasms, epithelial neoplasms, and no other specified carcinomas."

**3.** ***Progression of colon cancer and metastasis are dependent, in part, on angiogenesis. In this respect, SFRP4 inhibits endothelial cell migration and the development of sprouts and pseudopodia as well as disrupts the stability of endothelial rings in addition to inhibiting proliferation. sFRP4 interfered with endothelial cell functions by antagonizing the canonical Wnt/β-catenin signaling pathway and the Wnt/planar cell polarity pathway. Most importantly, SFRP4 restricted tumor growth in mice by interfering with endothelial cell function. The data demonstrate sFRP4 to be a potent angiogenesis inhibitor that warrants further investigation as a therapeutic agent in the control of angiogenesis-associated pathology (A. Muley et al. (Secreted Frizzled-Related Protein 4 An Angiogenesis Inhibitor. Am J Pathol. 2010 Mar; 176: 1505–1516). The authors should discuss this aspect.***

**Response:** As suggested by the reviewer, the aspect of the role of SFRP4 in angiogenesis was added to the discussion section of the manuscript.

"Angiogenesis plays an important role in colon cancer progression and metastasis. Study of Muley at al. has shown that SFRP4 can restrict ovarian serous cystadenocarcinoma growth in mice by interfering with endothelial cell function suggesting SFRP4 as a potent angiogenesis inhibitor. This is in contrary with our observations of SFRP4 upregulation in early-onset colon cancer that tends to be more aggressive and its strong correlation with epithelial mesenchymal-linked genes. These observations suggest that SFRP4 mechanisms of action might vary between different cancer types."

4. ***The manuscript contains too many abbreviations, which make it difficult to read.***

**Response:** We would like to thank the reviewer for this comment. We have spelled out majority of abbreviations in the manuscript what should provide more clarity and make it easy to read.

***5. The authors should provide a diagram summarizing their concept how SFRP4 expression causes poor survival in colon cancer.***

**Response:** We would like to thank the reviewer for this comment. This was merely an exploratory study with the goal of looking whether there is a correlation between expression of SFRP4 (found to be significantly upregulated in more aggressive early-onset colon cancer) and EMT-linked genes with regard to patients' survival using a larger publicly available expression data from TCGA database. More extensive experiments will have to be performed to draw a conclusion that SFRP4 up-regulation is causing poor survival in colon cancer patients.

In a summary, we have now addressed all comments raised by the reviewers. We would like to thank all reviewers for their insightful reviews. We look forward of having our manuscript accepted by the **World Journal of Gastrointestinal Oncology**.

Best Regards,

Jana Jandova, PhD
Landry Nfonsam, PhD
Valentine Nfonsam, MD