

February 15, 2019.

**Title: 'Up-regulation of TNF- $\alpha$  pathway survival genes and of the receptor TNFR2 in gastric cancer'**

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**Manuscript NO:** 45616

### STATEMENT

Dear Sir Editor-in-Chief, Lian-Sheng Ma - World Journal of Gastrointestinal Oncology

Thank you for your letter dated January 28, 2019 with the overall positive comments concerning our manuscript, and the request that we resubmit a revised version of it.

As you will see from the resubmitted copy we have addressed the issues raised by the Referee in a constructive manner, clarifying some issues. Below we list our responses to each individual point raised by the Referee and by the editor.

**Comments: Reviewer's code: 03966983**

This study by Rossi et al. reported that the expression of important genes of the TNF- $\alpha$  signaling pathway were up-regulated in gastric cancer. They concluded that this may promote cell survival possibly by TNF- $\alpha$ /TNFR2/NF $\kappa$ B pathway and negatively controls TNFR1-mediated apoptosis, which is highlighted by predominant expression of anti-apoptotic in relation to pro-apoptotic mediators. As the authors mentioned this is the first study to investigate the expression of genes that participate in the TNF $\alpha$  pathway and its relationship to miRNA expression in gastric cancer, which could open up new realms of interrogation. For this one, very important reason I feel that the work deserves publication.

Below, I provide a minor comment which I hope will improve the manuscript. In the last paragraph of the introduction, it is better to write more clearly about the aims of the study. I recommend to be re-written.

**Reply to the Reviewer:**

We thank the Reviewer for their comments about the manuscript and its careful analysis. As your comment, we rewrite the last paragraph of the introduction and believe that the aims of study are now clearer.

**Comments: Reviewer's code: 02982391**

World Journal of Gastrointestinal Oncology Up-regulation of TNF- $\alpha$  pathway survival genes in gastric cancer via TNFR2 ID: 01047678. I read with great interest the above titled article. However, there are several problems in the manuscript.

We thank the Reviewer for their comments about the manuscript and its careful analysis.

1. Abstract: The title of the article is not reflective of the aims of the study.

**Answer:** To answer the reviewer's comment the title has been changed to: **'Up-regulation of TNF- $\alpha$  pathway survival genes and of the receptor TNFR2 in gastric cancer'**

2. Abstract: Methods: is not well-described- the first three lines are factual information.

**Answer:** The description of the methods was rewritten as suggested by the reviewer.

3. Abstract: What do you mean by "revealed various possible relationships"?

**Answer:** From the construction of the miRNA: mRNA interaction network it was possible to observe that the miRNAs evaluated in this study target several of the TNF-  $\alpha$  pathway genes, thus showing several interrelations between the miRNAs and their target genes and between gene:gene, as can be observed in Figure 3. This sentence was changed in the abstract to be clearer.

4. Abstract: conclusions are not based on cause effect relationships. The relationship is not strongly defended in the experimental work.

**Answer:** We understand the reviewer's comment on the conclusions of our study, as no functional experiments have been performed to prove this cause-effect relationship between high TNFR2 receptor expression and the cell survival pathway. However, our results clearly show that the increased expression of TNFA / TNFR2 / NF-kB is related with the elevated expression of cell survival genes, and conversely, low expression of apoptosis pathway genes, such as the TNFR1 receptor and CASP3. Therefore, both conclusions of the abstract and the manuscript were rewritten.

5. Introduction: First two lines- but there are global geographical variabilities regarding the incidences of gastric cancer. The statement is too broad.

**Answer:** We agree with the reviewer's comment on the broad geographic variation of gastric cancer incidence and this information was added in the first paragraph of the introduction, as well as its incidence in Brazil.

6. Introduction: What was the problem? What was the rationale of the study? What were your research questions and your hypothesis? It is not clear from what stated in the last 2 paragraphs how this work fits with earlier work.

**Answer:** We understand the issues raised by the reviewer and rewrite the last paragraph to make clearer the reason that led us to do this work and the relationship with our previous work, published by Rossi et al. 2016. These clarifications have also been added in the article highlights (**Research motivation**)

7. Materials and Methods: Start with a study design: Briefly describe how your methods will enable you to answer your research question(s). State the number of samples (number of patients), sources of samples, etc. Were the 31 samples from 31 patients?

**Answer:** We agree with the reviewer's comment and added the subtitle "Clinical Samples" as the first item of Materials and Methods. In this subtitle, the information regarding the samples and patients were described in more detail.

8. Statistical analysis- give a reference for methods used in statistical work.

**Answer:** We agree with the reviewer's comment and added the reference number 38 (Daniel WW; Cross CL. Biostatistics: A Foundation for analysis in the health sciences. 10<sup>th</sup> ed. Jon Wiley & Sons, Inc., 2013) used to choose the best statistical tests.

9. Results: Maintain the subtitles under results to mirror those under methods.

**Answer:** We understand the issues raised by the reviewer and added the subtitle "Molecular diagnosis for *H. pylori*", besides rewrite the last subtitle to "miRNA-mRNA correlation and interaction networks", in order to present a better match between Methods and Results.

10. The authors may need to read these studies (none were cited): Zabaglia LM et al (2018), Zhang X et al (2013), Zhou C et al (2013).

**Answer:** We understand the issues raised by the reviewer and read the suggested articles. Findings on the miR-181c expression in gastric cancer samples observed by Zabaglia et al (2018) were included in the discussion. With regard to other articles, although they are very valuable studies that have brought important results on the miRNA / gene relationship, we did not add in the discussion since neither miRNAs miR-372 and miR-373 nor the gene TNFAIP1 were evaluated in the present study.

11. Conclusions: looks different from the conclusion stated in the abstract. I cannot see a cause effect relationship from experimental work conducted. The authors may need to be careful with their statements.

**Answer:** We understand the reviewer's comment on the conclusions of our study and as well as for the abstract's conclusion, conclusion of the manuscript was rewritten.

**Reply to the Science Editor:**

The manuscript was carefully revised as per the journal's instructions. The changes made to the manuscript have been highlighted in yellow. Moreover, we provide the decomposable and editable figures and wrote the article highlights.

Yours sincerely,

Ana Elizabete Silva

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