UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de São José do Rio Preto

February 21, 2022.

Title: "Downregulation of TNFR2 decreases survival gene expression, promotes apoptosis, and affects the cell cycle of gastric cancer cells"

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STATEMENT

Dear Sir Editor-in-Chief, Andrzej S Tarnawski – World Journal of Gastroenterology

Thank you for your letter dated February 07, 2022 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.

Reviewer #1: it is very well written the manuscript includes everything.

Reply to the Reviewer #1**:**

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

Reviewer #2: - please provide list of abbreviation. - the authors should give details about characteristics of H. pylori strain that be applied for this study such as ATCC, or drug susceptibility pattern, virulence factor etc. - please explain details of how eHP was produced. - the author need to be provided a complete list of primers with thermal cycle programs that be used for Quantitative PCR.

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Reply to the Reviewer #2**:**

1- Please provide list of abbreviation.

Answer: As requested by the reviewer, the list of abbreviations was made and follows below. However, as it is not requested in the WJG guidelines, we did not include it in the manuscript. If necessary, we can add it later.

List of abbreviations
GC – Gastric Cancer
H. pylori – Helicobacter pylori
TNF - tumor necrosis factor
TNFR1 – tumor necrosis factor receptor 1
TNFR2 – tumor necrosis factor receptor 2
eHP - *Helicobacter pylori* extract
miRNA – microRNA
TRADD - TNF receptor type 1-associated death domain protein
NF- κB – Nuclear factor κapa Beta
cIAP - cellular inhibitor of apoptosis proteins
TRAF2 - TNF receptor associated factor
cFLIP - CASP8 homologue FLICE-inhibitory protein
FADD - Fas-associated death domain protein
shTNFR1 – AGS cell lines with downregulated of TNFR1
shTNFR2 - AGS cell lines with downregulated of TNFR2
shRNA – short hairpin RNA
FITC - Annexin V- fluorescein isothiocyanate
PI - propidium iodide
AGS-C – Non-silenced AGS cell line control
AGS-eHP – Non-silenced AGS cell line treated with *Helicobacter pylori* extract
shTNFR1-C – AGS cell lines with downregulated of TNFR1 control
shTNFR1-eHP – AGS cell lines with downregulated of TNFR1 treated with *Helicobacter pylori* extract
shTNFR2-C – AGS cell lines with downregulated of TNFR2 control
shTNFR2-eHP – AGS cell lines with downregulated of TNFR2 treated with *Helicobacter pylori* extract

2- The authors should give details about characteristics of *H. pylori* strain that be applied for this study such as ATCC, or drug susceptibility pattern, virulence factor etc. - please explain details of how eHP was produced.

Answer: We agree with the reviewer's comment and this information was added in **MATERIAL AND METHODS - *Treatment with H. pylori extract (eHP)*** (page 7).

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"The previously described *H. pylori* Tox+ strain (cagA+/vacA s1m1) was grown in a selective medium (pylori-Gelose; BioMérieux, Marcy-l'Étoile, France) at 37°C under microaerophilic conditions (Santos et al., 2018, Am J Pathol 2018, 188: 329e335; https://doi.org/10.1016/j.ajpath.2017.10.005). This strain is not resistant to any antibiotic used to treat Hp. *H. pylori* extract (eHP) was prepared according to the protocol described by Li et al. (2007)[15]. In summary, *H. pylori* Tox+ strain was harvested and suspended in distilled water at a concentration of 2 x 10^8 CFU/mL. Next, the suspension was incubated at room temperature for 40 min and centrifuged at 20 000 g for 20 min. The supernatant was filtered using a 0.2 mm filter, and stored at -20C until used. "

3. The author need to be provided a complete list of primers with thermal cycle programs that be used for Quantitative PCR.

Answer: For gene expression and microRNA analysis, we used predesigned, commercially available Taqman assays (Applied Biosystems). These assays are designed and validated by the customer, which does not make the sequences of probes and primers available for commercial reasons; however, each assay receives an identification code (Hs, ID or catalog number), provided in the table below, which allows the exact identification of each assay used in the study. This information was added to the manuscript in the Materials and Methods section – *Quantification of mRNA and miRNA expression by RT-qPCR* (page 10).

Gene or microRNA	Identification code
TNF	Hs01113624_g1
TNFR1 (TNFRSF1A)	Hs01042313_m1
TNFR2 (TNFRSF1B)	Hs00961749_m1
TRADD	Hs00182558_m1
TRAF2	Hs00184192_m1
CFLIP (CFLAR)	Hs00153439_m1
NFKB1	Hs00765730_m1
NFKB2	Hs01028901_g1
CASP8	Hs01116281_m1
CASP3	Hs00234387_m1
ACTB	Catalog#: 4352935E
GAPDH	Catalog#: 4352934E

hsa-miR-19a-3p (MIMAT0000073)	ID000395
hsa-miR-34a-3p (MIMAT0004557)	ID 002316
hsa-miR-103a-3p (MIMAT0000101)	ID 000439
hsa-miR-130a-3p (MIMAT0000425)	ID 000454
hsamiR-181c-5p (MIMAT0000258)	ID 000482
RNU6B	ID 001093
RNU48	ID 001006

Regarding the thermal cycle programs used for quantitative PCR, we used the default cycling for all assays we use default equipment cycling conditions (95ºC for 10 minutes followed by 40 cycles at 97ºC for 15 seconds and 60ºC for 1 minute), following the manufacturer's instructions.

Yours sincerely,

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