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De-Ling Kong, PhD, Professor
Editor-in-Chief
World Journal of Experimental Medicine

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 014444-revised.doc).

Title: Role of the Wnt/β-catenin pathway in gastric cancer: An in-depth literature review

Author: Miguel Angel Chiurillo

Name of Journal: *World Journal of Experimental Medicine*

ESPS Manuscript NO: 14444

The manuscript has been improved according to the suggestions of reviewers:

1. Format has been updated and highlighted in yellow major changes concerning the suggestions of the reviewers. However, major changes were made in the organization and writing of the entire manuscript.

2. Revision has been made according to the suggestions of the reviewer:

Reviewer 00066723

Major comments
1. Please check English grammar throughout the manuscript paying particular attention to the abstract.
R: The writing and English was revised extensively throughout the text of the manuscript.
2. Page 3, line 25 – "proliferation / stem cell" can hardly be considered an oncogenic pathway. Please correct.
R: It is true. It was eliminated the word oncogenic, remaining as "signaling pathways". Page 5.
3. Figure 3 – from CK1α and GSK3β inhibitory lines run to β-catenin (in off-state panel) and to LRP5/6 (in on-state panel). This is confusing as CK1α and GSK3β phosphorylate (not inhibit) β-catenin and LRP5/6. Please check and change.
R: Figure 1 was changed according to the suggestion.
Minor comments

1. Page 4, line 22 – "ciliogenes" what exactly is meant ciliogenesis? Please check.
R: It was changed to ciliogenesis. Page 6.
2. Page 5, line 2 – Which "interaction" is meant here? Please rephrase sentence to make this more clear.
R: The sentence was rephrased to specify that refers to the interaction forming complexes between cadherin and catenin proteins. Page 6.
3. Page 5, line 24 – Use proper gene abbreviation throughout the text e.g. CCND1 instead of cyclin D1.
R: The text was revised to use the correct abbreviation for the mentioned genes.
4. Please include appropriate reference in Table 1
R: Table 1 was improved and appropriate references were placed.
5. Page 7, line 2 – For the casual reader it is difficult to understand what exactly the author tries to convey by "….wnt-6 is a critical mediator of the resisitance of gastric cancer cells to anthracycline drugs promoting by caveolin-1" Please rephrase sentence and explain in more detail.
R: Sentence was rephrased and explained with more detail. Page 9.
6. Page 9, line 29-30 – "Meanwhile, HMGA2 causes EMT by suppressing the expression of AXIN1 and increased TWIST1 expression through the binding to their promoters." Sentence does not read well, please rephrase. Also the next sentence is somewhat ambivalent, please change.
R: These and other sentence with similar problems were rephrased and explained with more detail throughout the text.
7. Page 10, line 12 and following – "CagA-independent manner" please explain what CagA is, for the reader not familiar with H. pylori this is unclear and hard to understand.
R: Some paragraphs were included for explaining *H. pylori* infection, its virulence factors and the importance in the origin of gastric cancer. Pages 20-21.
8. Page 16, line 2 – "consecuense" should this be "consequence"?
R: It was changed to "consequence". Page 23.

Reviewer 02441737

It is a very interesting manuscript; although t is recommended that the authors note in the title that this is a review of the literature focuses on findings on Wnt/β-catenin pathway as it relates to gastric cancer.
R: the title was modified to indicate that the manuscript is a review of the literature.
It is encouraged to author if possible, dividing the manuscript in subtitles; i.e.separating sections in which one by one the steps of the signaling via Wnt / β-catenin pathway proposed as the origin of gastric cancer.
R: I appreciate the suggestion of the reviewer, but in this sense I decided to follow the change proposed by the reviewer *00255764*, as well as the inclusion of other subtitles according to new information added by suggestion of reviewers.
It is also recommended, that the author present in the introduction, details inpercentage terms of the magnitude (prevalence of gastric cancer and itsconsequences) locally (Italy), in Europe and around the world.
R: I added some statistics information about gastric cancer incidence and mortality worldwide in the introduction section. Page 4.
If possible comment on the document the role of the physical and psychologicalstress in the activation of the Wnt / β-catenin pathway in gastric cancer.
R: Unfortunately it was not possible to obtain published information concerning "the role of the physical and psychological stress in the activation of the Wnt/β-catenin pathway in

gastric cancer" (despite performing an exhaustive search, therefore it was not included anything about it.
Table 1, it is not clear or at least for my document is not, it is advisable to present it more clearly.
R: R: Table 1 was improved and appropriate references were placed.

Reviewer 00255764
Major comments:
1. The readability of the manuscript suffers from a number of grammatical errors, awkward sentences and some clumsy turns of phrase. The abstract needs particular attentions as this probably the most important piece of text to grab a reader's attention. Try to make it clearer and more concise.
R: The writing and English was revised extensively throughout the text of the manuscript.
2. Early on in the manuscript a brief overview (and potentially a figure) of the histological/pathological development associated with gastric cancer initiation/development/progression would help put the cellular and molecular work discussed into context.
R: I included brief information about the model for the development of intestinal-type gastric cancer. Pages 4,5.
3. A brief introduction to H.pylori and it major virulence factors would again help provide some context. Is H.pylori infection important for initiation, development or maintenance of gastric cancers? What is CagA and how it might function (transcriptional regulator, kinase etc?
R: Some paragraphs were included for explaining *H. pylori* infection, its virulence factors and the importance in the origin of gastric cancer. Pages 20-21.

Minor comments or suggestions:
1. There are too many intriguing, but poorly developed points that leave the reader "hanging". For example: "This action of TC1 is achieved relieving the antagonistic function of Cby on ?-catenin-mediated transcription[76]." What is Cby and how does it antagonise B-catenin-mediated transcription? Should Cby's mechanism be explained in the "pathway antagonist" section? "Consequently, this induces aberrant expression of the intestinal-differentiation marker goblet-cell mucin gene MUC2 [82]." What is MUC2 and how does it influence tumour progression or Wnt signalling. Does it act in the same way as MUC1? "… E3 ubiquitin-protein ligase RNF138, which negatively modulates Wnt/?-catenin signaling pathway, was downregulated by H. pylori[86,87]." How does RNF138 inhibit Wnt signalling? These and the other examples need to be explained/developed further.
R: These and other points with similar problems were rephrased and explained with more detail throughout the text.

2. The general introduction to Wnt signalling would benefit from a section on the regulatory mechanisms governing B-catenin import / cytoplasmic sequestration.
R: I included a paragraph about mechanisms that regulates the accumulation of β-catenin in the cytoplasmic and nuclear compartments. Page 7.
3. I would suggest distilling down the "genomic alterations" section and incorporating it into new subheadings concerning "Gain of Wnt activator function in gastric cancer" or "Loss of Wnt repressor function in gastric cancer" sections.
R: I agree with the reviewer and assumed the suggestion.

4. The existing text in the "Wnt ligands" and "Wnt antagonist" sections can also be incorporated into the "Gain" and 'Loss" sections. Within each section the author can have

"Genetic, epigenetic and post-translational" subheadings. Together this structure should make the review more coherent.
R: I agree with the reviewer and assumed the suggestion. However, section about "Role of microRNAs in Wnt/ß-catenin signaling in the gastric cancer" was kept apart due to in many cases the exact mechanisms of action on controlling Wnt signaling aren't known.

5. Removal of the passive voice ("that have been shown… etc")
R: I eliminated a lot of use of the passive voice in the manuscript.

6. I would explicitly point out some of the apparent paradoxes in Wnt signalling, as well as potential explanations. For example: Wnt negative feedback mechanisms normally prevent rampant, unrestrained Wnt signalling, but selective repression of certain Wnt target genes may uncouple the negative feedback.
R: I agree with the reviewer. I added information concerning the negative feedback generated by Wnt antagonists that also are transcriptional targets of the pathway. I also made a comment about the mechanisms by which tumor cells can overcome this inhibition. Page 12.

7. What is the actual mutation frequency of CTNNB1 in gastric cancer? It is not clear from the following sentence. Is it 30% of all gastric cancers or 30% of those gastric cancers identified with mutant CTNNB1? "Some mutations in CTNNB1, the gene that encodes β-catenin protein, have been frequently detected in intestinal- and diffuse-type gastric cancer with 30% of tumors showing nuclear accumulation of β-catenin[7,95,99-103]."
R: The sentence was corrected. It means that 30% of gastric carcinomas with nuclear accumulation of β-catenin have mutations in *CTNNB1*. Page 10.

Reviewer 00646379

Need to perform some editing and following references should be included for clarity of the field. Dig Dis Sci. 2013 Mar;58(3):724-33. Int J Oncol. 2011 May;38(5):1375-83. Int J Mol Med. 2014 Jul;34(1):197-204 Eur J Cancer. 2013 Nov;49(17):3718-28 Cancer Lett. 2013 Oct 28;340(1):72-81. Oncol Rep. 2013 Sep;30(3):1137-42. Mol Med Rep. 2013 Jun;7(6):1751-6. Tong et al. Oncotarget. 2014 Sep 25. [Epub ahead of print] Zhang et al. Proton pump inhibitor pantoprazole abrogates adriamycin-resistant gastric cancer cell invasiveness via suppression of Akt/GSK-β/β-catenin signaling and epithelial-mesenchymal transition. Cancer letters, DOI: 10.1016/j.canlet.2014.10.016

R: All references mentioned by the reviewer were included and discussed in appropriate sections of the manuscript

Reviewer 00531670
In my view the manuscript would be suitable for publication following some minor revision.
1. Association of the Wnt pathway with gastric stem cells needs to be addressed thoroughly
R: I included a section about generalities of gastric stem cells and the role of the Wnt signaling pathway in the development and renewal of this cells. Page 17.

2. The section on the involvement of the miRNAs in the control of the Wnt pathway in gastric cancer cells is very short and should be extended.
R: I extended the information about the role of microRNAs in Wnt/ß-catenin signaling in the gastric cancer. Page 15.

3. References and typesetting were corrected

Thank you again for publishing my manuscript in the *World Journal of Experimental Medicine.*

Sincerely yours,

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