ANSWERING REVIEWERS

7th July 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: garg_minal revised manuscript for WJSC).

Title: Epithelial-mesenchymal transition – activating transcription factors – multifunctional regulators in cancer

Author: Minal Garg
Name of Journal: *World Journal of Stem Cells*

ESPS Manuscript NO: 3719

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

2. Revision has been made according to the suggestions of the reviewer. Please find below the comments by the reviewers and response to the comments.

Comments by reviewer 1:

The manuscript entitled "Epithelial-mesenchymal transition – activating transcription factors – multifunctional regulators in cancer" is a concise but rather comprehensive review about the transcription factors and the signaling pathways involved in the regulation of Epithelial-mesenchymal transition. Major point: The manuscript should undergo thorough English language editing before publication. Minor point: At least in my version of the manuscript, Table 1 is split into two parts, which should be pasted together.

Response to the comments by reviewer 1

The manuscript has been revised. Editing for English language was done wherever required. Table 1 is not split into many parts. It represents induction of various hallmarks of cancer due to increased expression of EMT-ATFs.

Comments by reviewer 2:

The general goal of this review paper is to provide a snapshot of our current understanding of some of the major players and mechanisms activating transcription factors (ATF) in the process of epithelial to mesenchymal transition (EMT), and how it relates to cancer. The author seeks to describe the activation of several signaling pathways that may lead to upregulation of EMT-ATFs, which are suggested to be important during physiological and pathological conditions. I have identified some major issues that should be addressed before the paper is accepted for publication. General comments: 1- The title could be improved so that it follows a more conventional format, rather than "enumerating" multiple ideas. 2- The author is encouraged to avoid enumerating proteins, function, regulatory pathways, and rather to provide a description of the aforementioned issues. 3- The style and grammar of the paper needs major improvements. Some sentences are very well written, and many others require major correction. For example, page 3, paragraph 2, sentence 5 is very confusing and should be revised. 4- Description of transcription factors and regulatory mechanisms is very limited or poorly described. 5- It will be helpful to include a final section describing what the author consider could be future avenues of research in this area.

Response to the comments by reviewer 2

Since this paper mainly describes the involvement of EMT-ATFs in pathways' regulations which recruit the various hallmarks of cancer, I chose this title. Editing for grammatical errors has been taken care of wherever necessary. Page 3, para 2, sentence 5 is revised. Transcription factors and their regulatory mechanisms are described in more detail. Few sentences on the future avenues in this research area are being added up as suggested by the reviewer.

Comments by reviewer 3:

The author has covered all the objectives set by the journal. However, my comments are as follows. 1) In the paragraph 2.1.1 titled ZEB1 and ZEB2, the author says that miR200a inhibits ZEB factors by a reciprocal negative feedback loop. Could the author explain when this negative loop stop functioning? 2) In the paragraph 3.3 "Wnt/βcatenin pathway", in my opinion the author should rewrite in a clearer way the section from "During canonical signaling as a result of Wnt binding…" to "…complex leading to targeted gene transcription". 3) All pathways mentioned in the paragraph 3.3 "Wnt/βcatenin pathway" are not well illustrated in figure 1. 4) In the paragraph 3.4 "Hedgehog pathway" the author writes that the activation of transcription factors GLI-1,-2 and -3 lead to transcription of GLI target genes. What are these target genes? What function(s) do they have? 5) In the figure legend, in my opinion the author should clarify that the binding of the EMT-ATFs to E box of promoter regions of epithelial genes have the function to inhibit these genes. 6) Pag. 21 Figure legends should be Figure legend. 7) The author has to check the format of references cited in the text – these have to be the same throughout the text. 8) The TNF-α and TNF-β pathways are not shown in the figure. Why? English language editing is required

Response to the comments by reviewer 3

1. Mir-200 controls the epithelial phenotype in various cancers. In epithelial cell lines, miR-200 expression is silenced by hypermethylation during TGFb-induced EMT but reverted by demethylation in MET. In mesenchymal-like ovarian cancer cells, it targets Zeb1 and Zeb2 factors and finally induce mesenchymal to epithelial transition phenotype. Invading mesenchymal-like cancer cells that extravasate and metastasize eventually need to revert to an epithelial phenotype for the metastatic colony to grow into a secondary tumor. Cancer cells that form macroscopic metastasis have higher levels of miR-200 compared to those that invade but are not able to colonize and, paradoxically to their roles as EMT repressors.
2. Paragraph 3.3 has been rewritten.
3. Wnt/ βcatenin pathway is shown in the fig. 1.4.
4. According to one of the studies sited here-cDNA microarray analysis results in the identification of 278 upregulated and 59 downregulated genes upon Gli1 expression in pancreatic cancer cells [Xu X, Zhou Y, Xie C, Wei SM, Gan H, He S, Wang F, Xu L, Lu J, Dai W, He L, Chen P, Wang X, Guo C. Genome-wide screening reveals an EMT molecular network mediated by Sonic hedgehog-Gli1 signaling in pancreatic cancer cells. PLoS One. 2012;7(8):e43119.]
   Markers of mesenchymal cells are upregulated and of epithelial cells are downregulated, could be examples of few target genes following the activation of GLI-1, 2, 3 transcription factors.
5. Fig. 1.4 is modified.
6. 'Figure legend' is corrected.
7. Format of references checked
8. Fig. 1 is revised.
9. English editing is done wherever required.

Comments by reviewer 4:

The review discusses a very interesting and current topic. The author focuses on the role of EMT in cancer-ATFs, analyzing the mechanisms of signal transduction pathways that lead to their transcription. However, some steps are not sufficiently explained and may not be understood by the reader, for example, E-cadherin, explain the role as a phenotypic marker of epithelial cells. The author clearly does not discuss the role of p53 and miR-200 and stem cell factors (Sox2 and Klf4) in modulating the expression of ATFs. In addition, the bibliography is not updated with recent work on the subject. Moreover in the section conclusions the author does not mention the use of specific drugs for ATFs modulation as reported in recent articles.

Response to the comments by reviewer 4

A brief discussion on tumor suppressor functions, stem cell maintenance, expression of ATFs and EMT has been included. Most of the references cited are from the past five years. Many of them are form 2012 and 2013. Conclusion section is modified.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Stem Cells*

Sincerely yours,

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