ANSWERING REVIEWERS

January 13, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 6867-review.doc).

Title: Cancer stem cells: involvement in pancreatic cancer pathogenesis and cancer therapeutics perspectives

Author: Cristiana Pistol Tanase, Ana Iulia Neagu, Laura Georgiana Necula, Cristina Mambet, Ana-Maria Enciu, Bogdan Calenic, Maria Linda Cruceru and Radu Albulescu

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 6867

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

Reviewe 1

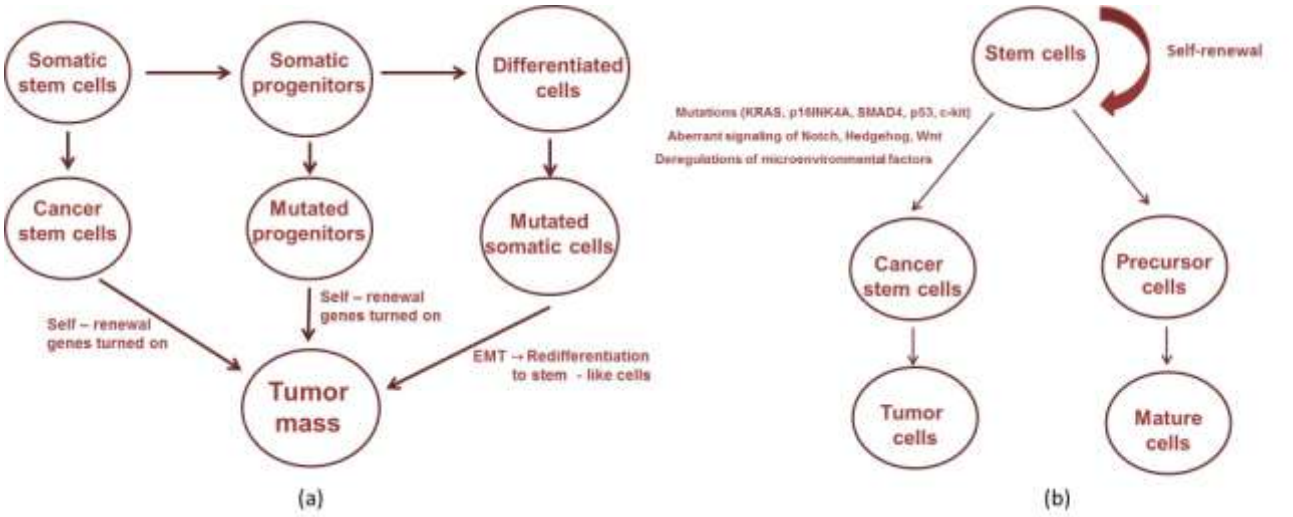
The paper by Cristiana Pistol Tanase et al reviewed pathogenesis and therapy of pancreatic cancer **focused on the cancer stem cell (CSC) model. The paper deserve attention due to the extensive and clear revision. However, there are points to clarify. Major comments: 1) The paradigm of cancer-initiating stem cells has initially been developed with respect to blood cancers where chronic conditions such as myeloproliferative neoplasms are due to mutations acquired in hematopoietic stem cells [7] The concept of CSCs and cancer-initiating cells (cells of origin) are two separate concepts. Indeed as reviewed in Nature by Visvadier JE (Nature 2011): cancers of distinct subtypes within an organ may derive from different 'cells of origin'. These cells acquire the first genetic hit or hits that culminate in the initiation of cancer. It is important to note that the cell of origin, the normal cell that acquires the first cancer-promoting mutation(s), is not necessarily related to the cancer stem cell (CSC), the cellular subset within the tumour that uniquely sustains malignant growth. That is, the cell-of-origin and CSC concepts refer to cancer-initiating cells and cancer-propagating cells, respectively. Although the tumourinitiating cell and the CSC have been used interchangeably, thetumour-initiating cell more aptly denotes the cell of origin. Please consider this clear and important distintiction and specify accordingly in the paper.**
We thank the reviewer for his/her comment and we do agree that the origin of a tumor is still a matter of debate and the concept of cancer stem cells is a controversy.
We used the indicated citation to clarify the concept of CSCs as required, using the following sentence at the end of Introduction section "Whether the cell of origin is a stem cell, a precursor or an adult cell is still a matter of debate and possibly, the "cell of origin" is responsible for the tumor phenotype and the relationship with cancer stem cells (1).
However, when using the term "tumor-initiating cells", we meant a subpopulation of cells able to generate tumors in in vivo models, feature that is specific for CSC side populations. Other authors, that recently published original articles in prestigious publications, used this term with the same connotation as us, e.g.:

- Cabarcas et al., Pancreas. 2013 Jul;42(5):861-70. doi: 10.1097/MPA.0b013e318279d568.
- Kim et al. PLoS One. 2013;8(1):e51671. doi: 10.1371/journal.pone.0051671. Epub 2013 Jan 2.
- Lo et al. Oncogene. 2012 May 24;31(21):2614-26. doi: 10.1038/onc.2011.439. Epub 2011 Sep 26.

We do respect, however, the reviewer's point of view, so we shall discard the term tumour-initiating cells from our manuscript and use, whenever appropriate, the term "side population" or other syntagm to indicate stem potential.

2) Figure 1 Factors involved in occurence of cancer stem cells. The emergence of mutations and aberrant signaling in normal stem cells, progenitors or differentiated cells triggers the transformation of normal cells into cancer stem cells, losing control of cell division. This figure does not reflect clearly the CSC model. Indeed, it does not emerge any gerarchy existing among the cells in the tumour mass that is a key finding of this model. Moreover also it does not emerge the mechanisms determining the inter-tumoral eterogeneity of the cancers that based on the cells of origin. Indeed wheter a tumor will originate from a stem cells it will determine a more aggressive tumor phenotype with respect a tumor originating from mature cells (see Visvadier JE Nature 2011)

We thank the reviewer for the comment, we modified the figure to also illustrate the hierarchy model



and modified the legend accordingly:

Figure 1 Models that explain tumor development. (a) The stochastic model states that every cell has the potential to be the "the cell of origin" of a tumor; (b) The "cancer stem cell hypothesis", proposes that tumor evolution is based on stem cells with 'deregulated' signalling pathways

Minor comments:

1) The main risk factors for pancreatic cancer include increasing age, smoking, chronic pancreatitis, diabetes mellitus, metabolic syndrome, low levels of serum vitamin D, family history of pancreatic cancer and rare inherited genetic conditions such as Peutz-Jeghers syndrome, familial melanoma and hereditary pancreatitis. Please indicathe references of this sentence.

We used references no:

[4] Leenders M, Chuang SC, Dahm CC, Overvad K, Ueland PM, Midttun O, Vollset SE, Tjonneland A, Halkjaer J, Jenab M, Clavel-Chapelon F, Boutron-Ruault MC, Kaaks R, Canzian F, Boeing H, Weikert C, Trichopoulou A, Bamia C, Naska A, Palli D, Pala V, Mattiello A, Tumino R, Sacerdote C, van Duijnhoven FJ, Peeters PH, van Gils CH, Lund E, Rodriguez L, Duell EJ, Perez MJ, Molina-Montes E, Castano JM, Barricarte A, Larranaga N, Johansen D, Lindkvist B, Sund M, Ye W, Khaw KT, Wareham NJ, Michaud DS, Riboli E, Xun WW, Allen NE, Crowe FL, Bueno-de-Mesquita HB, Vineis P. Plasma cotinine levels and pancreatic cancer in the epic cohort study. *Int J Cancer* 2012; **131**: 997-1002

[PMID: DOI: 10.1002/ijc.26452
[5] Greenhalf W, Malats N, Nilsson M, Bartsch D, Neoptolemos J. International registries of families at high risk of pancreatic cancer. *Pancreatology* 2008; **8**: 558-565 [PMID: DOI: 10.1159/000159214

2) High mortality rate of pancreatic cancer is due to difficulty in early diagnosis and its notorious resistance to chemotherapy and radiation. Please indicate the reference of this sentence.

To answer the reviewer's request, we used ref no [7] Long J, Zhang Y, Yu X, Yang J, LeBrun DG, Chen C, Yao Q, Li M. Overcoming drug resistance in pancreatic cancer. *Expert Opin Ther Targets* 2011; **15**: 817-828 [PMID: 3111812 DOI: 10.1517/14728222.2011.566216

3) Lack of clinical symptoms in early stages leads to delay in tumor detection; thus, approximately 80% of patients with pancreatic cancer have a metastatic disease at the moment of diagnosis. Please indicate the reference of this sentence.

To answer the reviewer's request, we used ref no [8] Bhat K, Wang F, Ma Q, Li Q, Mallik S, Hsieh TC, Wu E. Advances in biomarker research for pancreatic cancer. *Curr Pharm Des* 2012; **18**: 2439-2451 [PMID: 3408036, http://www.ncbi.nlm.nih.gov/pubmed/22372502

4) The paradigm of cancer-initiating stem cells has initially been developed with respect to blood cancers where chronic conditions such as myeloproliferative neoplasms are due to mutations acquired in hematopoietic stem cells [7]. It seems the ref is not appropriate with respect the citation

5) 24049451, 12629218, 22956869; What are the meanings of these numbers?

These are PMIDs for the respective citations. We apologize for this missformating of our references, it has been corrected in the revised manuscript.

6) Pag. 9 in vitro and in vivo in italic form

We performed the necessary correction, thank you.

7) Please describe briefly the anti-tumoral mechanisms of action of salinomycin.

The mechanisms proposed for the anti-tumor activity of salinomycin included: i) inhibition of Wnt/β-catenin signaling (2); ii) inducer of apoptosis and autophagy via AMPK activation (3); iii) increased DNA breakage and phosphorylated levels of p53 and H2AX (4); iv) cell cycle arrest and apoptosis via downregulation or inactivation of cell cycle-associated oncogenes, such as Stat3, cyclin D1, and Skp2 (5)

1. Jane EV. Cells of origin in cancer. Nature. 2011;469(7330):314-22.
2. He L, Wang F, Dai W-Q, Wu D, Lin C-L, Wu S-M, et al. Mechanism of action of salinomycin on growth and migration in pancreatic cancer cell lines. Pancreatology. 2013;13(1):72-8.
3. Zhu LQ, Zhen YF, Zhang Y, Guo ZX, Dai J, Wang XD. Salinomycin Activates AMP-Activated Protein Kinase-Dependent Autophagy in Cultured Osteoblastoma Cells: A Negative Regulator against Cell Apoptosis. PLoS One. 2013;8(12):e84175. Epub 2013/12/21.
4. Kim JH, Chae M, Kim WK, Kim YJ, Kang HS, Kim HS, et al. Salinomycin sensitizes cancer cells to the effects of doxorubicin and etoposide treatment by increasing DNA damage and reducing p21 protein. Br J Pharmacol. 2011;162(3):773-84. Epub 2010/10/27.
5. Koo KH, Kim H, Bae YK, Kim K, Park BK, Lee CH, et al. Salinomycin induces cell death via inactivation of Stat3 and downregulation of Skp2. Cell Death Dis. 2013;4:e693. Epub 2013/06/29.

3 References and typesetting were corrected

Reviewer 2

Revision has been made according to the suggestions of the reviewer

This is a good and thorough review focusing on cancer stem cells in the pathogenesis and therapies in pancreatic cancer. The contents are relevantly updated with appropriate references. There are two

minor topographical mistakes needed to be changes: 1. On page 10 last two lines the word remarcably should be "remarkably" and also "non-metastatic" is the right word. 2. Page 11, This process appears to "be" regulated.... And also ...is also "acted".. also on page 17, et all should be "et al." There may be other mistakes needed to be check through out the text.

We thank the reviewer for his/her kind appreciations and we operated the required modifications. We also checked the rest of the manuscript for additional errors.
References and typesetting were corrected

Reviewer 3
The authors summarized the research in the literature regarding cancer stem cells (CSCs) in pancreatic cancer pathogenesis and the role of CSCs as a potential target for pancreatic cancer therapy. Overall, it is an interesting topic and the whole article is well-organized. The manuscript provides useful information to researchers and clinicians working in the field of tumor/stem cell biology. The manuscript may be enhanced by including the relationship between the dysregulated microRNAs and CSC-like cells in pancreatic cancer. It will be helpful for the readers to have a fuller view of the MiRNAs regulators in pancreatic cancer.

We thank the reviewer for his/her appreciative words. By adding the following paragraph, we hope to meet te reviewer's requirements:
"More studies suggest that stem cells convert to cancer stem cells by deregulation of miRNAs expression which affect several signaling pathways involved in proliferation, apoptosis, and more important, renewal and differentiation of stem cells 22993663, 20635434. Nanog and Sox2, important regulators of stem cell pluripotency and CD44 stem cell surface marker are examples of the miRNAs targets. Alia Ahmed, Shadan Ali, Philip A Philip, Fazlul H Sarkar The Role of Cancer Stem Cells and MicroRNAs in the Development and Progression of Pancreatic Cancer Ahmed, J Stem Cell Res Ther 2012, S7
Using microarray analysis Jung et al showed that pancreatic cancer stem cells present a different expression of miR-99a, miR-100, miR-125b, miR-192, and miR-429 compared with control 21785383. In vitro study conducted on human pancreatic cancer cell lines AsPC-1, AsPC-1-GTR, MiaPaCa-2, and MiaPaCa-2-GTR showed that reexpression of miRNAs (let-7a, let-7b, miR-26a, miR-101, miR-200b, and miR-200c) which are normally lost in pancreatic cancer and especially in pancreatospheres can revert or destroy CSCs 22086681. Another study reports the loss of miR-34 in CD44+CD133+ MiaPaCa2 pancreatic cancer cells while miR-34 restoration led to inhibition of tumor-initiating cell population, of tumor cell sphere growth and of tumor formation 19714243, 24281178.
Wellner et al. demonstrated that miR-200c, miR-203 and miR-183 activity can lead to downregulation of stem cell factors, founding a regulatory feedback loop between miRNAs and CSC in pancreatic cancer 19935649
In this regard, understanding of miRNAs alterations can lead to the development of better strategies in treatment of pancreatic cancer patients by elimination of CSCs."

The references are added as PMID, for ease of use and included in the text as proper references.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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