Format for ANSWERING REVIEWERS

Sep. 24, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 2429-review.doc).

Title: Development of Cancer-Initiating Cells and Immortalized Cells with Genomic Instability

Author: Yuko Atsumi, Hitoshi Nakagama and Hirobumi Teraoka

Name of Journal: World Journal of Stem Cell

ESPS Manuscript NO: 12725

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

Reviewer 2446280

Response: We would thank to the reviewer for taking your time to read our manuscript and the favorable decision. Over the revision of the manuscript, we believe English written has improved.

Reviewer 1179981

COMMENTS FOR THE AUTHOR: Yoshioka et al., describe the role genomic instability plays in the development of cancer stem cells. They contrast MEF immortalization to CSC development. They claim the general process of genomic instability is the same but that MEFs do not possess qualities of stemness. In general there is insufficient description on key points.

Response: We would like to thank to the reviewer for the useful and insightful comments. By responding to each of the reviewer's comments, we have revised the text accordingly.

Comment: 1) Cancer stem cell is a confusing concept: "cancer"-initiating cell might work better.

Response: As requested, we have changed to "cancer"-initiating cell to minimize unnecessary confusion.

Comment: 2) There is no discussion about the DNA repair pathways that correct damage and the defects that can lead to hereditary cancer. There should be at least a brief mention of these pathways. A detailed description of these pathways and their relevance to hematopoietic stem cells was recently published and this paper should be referenced [1].

Response: As requested, we have added the argument of DNA repair deficient background (p5 line9-11), with some additional references that includes the manuscript pointed by the reviewer.

Comment: 3) Briefly describe the DNA repair pathways that suppress CIN and MSI.

Response: As requested, we have briefly added the DNA repair pathways that control CIN and MSI (p5 line7-9).

Comment: 4) The authors discuss mutations in p53 cause cancer and then mention that the DNA damage response might not be important for tumor suppression but fail to mention that p53's antagonism of glycolysis and/or the mTOR pathway are prime suspects. There are many good reviews on the topic and at least one should be referenced [2-6].

Response: As requested, we have added the argument of p53's antagonism of glycolysis and/or the mTOR pathway with the relevant references (p6 line22-23).

Comment: 5) The authors claim that MEF immortalization and CSC transformation are distinct processes because MEF do not possess stemness. However, in my experience immortalized MEFs always develop into cancer when injected subq. into isogenic mice. Please explain.

Response: We would thank to the reviewer to raise this point. We found that the way we wrote was not appropriate. We have revised for this issue (p8 line4-8).

As it has previously shown, immortalized fibroblast from rodent (including MEFs) do not efficiently develop tumor unless those are pre-transformed by oncogenes, such as Ras and Myc (Land et al., 1983; Newbold and Overell 1983). We now inserted the references as well. However, as the reviewer pointed, I know the immortalized MEFs still can form cancer in the current technique, in which the efficiency is very low compared to cancer initiating cells that can even to develop cancer from a single cell.

I believe that the process of secondary tumor development by immortalized MEFs is not really addressed. In this process, the spontaneous transformation might be involved in, because immortalized MEFs are clearly different from CICs, the cells responsible for cancer development. In fact, while CICs express pluripotent cell marker genes, form sphere, and require high glucose medium for the cultivation, immortalized MEFs do not.

Comment: 6) To my knowledge there is no evidence of a difference between immortalization and carcinogenesis because of Stemness. Please justify this idea using published data.

Response: We have to agree to the reviewer. It was not appropriate the way we had described, we have revised for this issue (p8 line4-12). The idea actually came up from a couple of backgrounds. 1st, unlike majority of other cancer cells, CICs posses a responsible role for cancer formation ability. 2nd, teratoma or teratocarcinoma can be developed even from normal ES cells, suggesting that stemness characteristics seem to be beneficial for tumorigenesity. 3rd, immortalized MEFs do not show stemness characteristics, which does not efficiently form tumor. Together, these lines of accumulating evidence suggest that one of the functions lacked in immortal MEFs to efficiently form tumor is a stemness characteristic.

Comment: 7) The authors give examples of experimental procedures that disrupt the niche. Please give examples of natural niche disruption that could lead to CSC development.

Response: We apologize for the confusion caused by the previous version of our manuscript. We have actually sited the relevant paper showing "natural niche disruption associated myelodysplasia and secondary leukaemia" (Raaijmakers, et al 2010 Nature 464, 852–857). This suggests CIC development by natural niche disruption because such tumor formation is dependent on CICs. This article directly suggests that stem cells differentiating in an aberrational environment are at a risk of cellular transformation into malignant counterparts. Other examples show the effects of the loss of niche and the resulting carcinogenesis (Knoepfler et al., 2009, Morange et al., 2006, Wylie et al., 2006). We have revised these issues (p9 line3-11).

Reviewer 2681588

Response: We would thank to the reviewer for taking your time with reading our manuscript and for the faithful comments.

Comment: Manuscript's title suggests that this review is going to approach wide concepts about CSC and GIN. However, the review basically describes the regulation of the ARF/p53 module and H2AX levels in cell immortalization. In order to be published either the manuscript should be revised and become more extensive or change and mention in title immortalization, p53 and H2AX. Figure is not representative of the text nor the title.

Response: Following the reviewer's comments, we have added a figure. However, title must be less than 12 words. Therefore, we could not mention p53 and H2AX in title and hence have carefully revised "summary" and "core tip" sections.

Comment: In abstract section, in the last phrase I suggest changing "Several lines of evidence" by "A recently proposed line of evidence" since this is based on the results of only one reference [45]. Please write a summary of less than 100 words to outline the most innovative and important arguments and core contends in your manuscript, in order to attract readers.

Response: Following the reviewer's comments, we rewrote the abstract and added the "core tip", which are more that 200 words and less than 100 words, respectively.

Comment: Citation [11] is from 1997, a recent paper should also be cited, such as Hveem TS et al. Prognostic impact of genomic instability in colorectal cancer. British Journal of Cancer, v.110, 2014. Citation is lacking in last phrase of introduction. Are the authors affirming, "the preponderance of evidence clearly shows that the majority of cancers develop in association with genomic instability induced in CSCs"? If so, they should better explain and cite more than only one work. It is not clear that MSI and CIN are present in CSC.

Response: We would thank to the reviewer. We added the reference suggested by the reviewer. We have also added some other manuscript associated with CIN and genomic instability in cancer. As the reviewer pointed, since "the last phrase of introduction" was not appropriated, which is now revised (p5 line6-12).

Comment: In "The effect of mutation in the ARF/p53 module" please add a brief comment about ARF such as "a tumor suppressor transcribed from an alternate reading frame of the cyclin-dependent kinase inhibitor 2A (CDKN2A) gene". Further description about H2AX also would be informative for the journal's readers. There are interesting papers related with this subject that were not considered in this manuscript: 1) Journal of Investigative Dermatology (2006) 126, 1214–1216. Genomic Instability and Tumor Stem Cells by James M Grichnik1 2) Connections between Genomic Instability and Cancer Stem Cells. From Linda Li, Laura Borodyansky and Youxin Yang 3) The paradigm of mutant p53-expressing cancer stem cells and drug resistance. Carcinogenesis. 2014 Jun;35(6):1196-208. Shetzer Y1, Solomon H1, Koifman G1, Molchadsky A1, Horesh S1, Rotter V2.

Response: We would thank to the reviewer. Following the reviewer's comments, we added the explanation of ARF and H2AX and now cited the manuscripts that were pointed by the reviewer with brief explanation (p5 line20-p6 line1 and p7 line9-11).

Minor issues: Running title would be: Genomic instability-associated CSC development They should use CD33+, CD133-, for example. The correct reference 12 is Shih IM, et al. Evidence that genetic instability occurs at an early stage of colorectal tumorigenesis. Cancer Res 2001; 61:818-822 [PMID: 11221861]

Response: Following the reviewer's comments, we have changed the running title and corrected the mistake in the reference.

Reviewer 02446105

Comment: Yoshika et al., presented a review article of the association of genomic instability in cancer stem cell development. Generally, this article should be interesting and may provide information regarding the association between the development of cancer stem cells (CSCs) and genomic instability. However, the author seems to address the associations only limited to ARF/P53. As it is a review article, a global picture of the potential genomic stabilities targeting on the CSC formation will be highly appreciated.

Response: We would thank to the reviewer for requiring global picture. As the reviewer pointed, the previous version was imbalanced in the regulation by the ARF/p53, which was because we mainly described recent mechanistic knowledge that was mainly studied in the systems in vitro. To revise this point, we have added a section for that, in which we described the role of other regulation factors and the difference from those of the roles of ARF/p53.

Comment: Major points 1. This review article tried to address the association between genomic instability and CSC formation but with limited information only look at ARF/P53. It has been well accepted that CSCs may be induced by three ways: (1) Directly transformed from the stem cells; (2) Reprogramming from the differentiated cancer cells with genomic instability; and (3) Reprogramming from the differentiated cancer cells under the factors of aberrant niche environment. The current manuscript put main efforts on part 1. A global map of the association between the genomic instability and CSC formation resulted from different ways is expected. to cover the concept of the current review contents.

Response: As pointed, we have briefly mentioned the other possibilities for the development of CICs (CSCs) with brief explanation. Concerning the regulation factors other than ARF/p53, this is tightly associated with above issue, hence revised the text together with addressing above comment (see above response).

Comment: 2. Minor points 1. The full name of specific term at the first description should be noted. For example: P3L14 ARF/P53 P4L19 H2AX P5L21 ES, iPS 2. English editing is suggested.

Response: Following the reviewer's comments, we have added the description for specific terms. We have sent to an English editing service.

3 References and typesetting were corrected

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

Sincerely yours,

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