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To Science editor
World Journal of Gastroenterology

Dear Dr. Qi,

We are very pleased with your decision requesting minor revision of our manuscript # 23334 entitled "***Eukaryotic elongation factor-1α 2 knockdown inhibits hepatocarcinogenesis by suppressing PI3K/Akt/NF-κB signaling***"

We also thank the reviewer who provided a comprehensive assessment of our work. The sound and insightful reviewer comments have helped us further improve our manuscript, we hope to your entire satisfaction.

Please find below a point by point response to all reviewer questions, remarks and suggestions.

Again, thanks for the opportunity to contribute to your journal.

Please do not hesitate if you have any further questions regarding this manuscript.

Best wishes,

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COMMENTS TO AUTHORS

1. In the Methods section, under subheadings "Lentivirus-based short hairpin RNA (shRNA) silencing in BEL-7402 cells and experimental grouping", "Real-time polymerase chain reaction (PCR)", and "Western blot" it is not mentioned for how long the BEL-7402 cells were cultured post-lentivirus infection, before being analyzed for gene expression.

Response:

Thanks to the reviewer for this critical point. BEL-7402 cells were cultured post-lentiviral infection for 5 days before gene expression analysis. This has been added in the indicated portions of the Methods. Please see page 7.

2. In the Methods section, under subheadings "Lentivirus-based short hairpin RNA (shRNA) silencing in BEL-7402 cells and experimental grouping" Last line of this subheading "Cells transfected at more than 80% were assessed" is confusing. Kindly clarify it further.

Response:

By "Cells transfected at more than 80% were assessed", we meant "Cells were used for subsequent experiments, when lentiviral transfection efficiency was above 80%". We apologize for the confusion. Please see page 6.

3. In Methods section, under subheading "Lentivirus-based eEF1A2 overexpression in SK-HEP1 cells and experimental grouping" lines 6-11 (pages 7/8) are a repetition of statement from previous subheading. These lines should be removed and information relevant to this subheading should be included here. Under same subheading, Line 2 of para 2 (page 8) the statement "with AgeI / AgeI double restriction enzyme digestion sites" does not look right.

Response:

This reviewer is absolutely right. We mistakenly described silencing instead of overexpression of eEF1A2 in SK-HEP1 cells. We have properly modified the indicated portion of the text. Please see page 6-7.

4. For MTT, cell proliferation, cell cycle, and apoptosis cells were cultured for 5 days while for colony formation assay they were cultured for 14 days. Kindly justify this difference with reference(s).

Response:

Thanks for this question. Cell proliferation, cell cycle and apoptosis are usually assessed during the first few days, since these events happen very fast in cells. Also, such assays are usually setup with relatively high cell numbers. In the case of colony formation assays, less cells are plated with the intention to have colonies, which takes long. Please see page 8.

A few references:

Wognum B, Yuan N, Lai B, Miller CL. Colony forming cell assays for human hematopoietic progenitor cells. Methods Mol Biol. 2013;946:267-83: 14 days for colony formation assay

Kim HK, Moon H, Lee KS, Moon HS, Kim BS, Kim DJ. Clonogenic assay of gastric adenocarcinoma stem cells--clonogenic assay, stomach cancer. Korean J Intern Med. 1987 Jul;2(2):163-9: 14 days for colony formation assay

Relja B, Meder F, Wilhelm K, Henrich D, Marzi I, Lehnert M. Simvastatin inhibits cell growth and induces apoptosis and G0/G1 cell cycle arrest in hepatic cancer cells. Int J Mol Med. 2010 Nov;26(5):735-41. Apoptosis, cell growth, cell cycle assessed at 24 to 72 hours.

Klucar J, Al-Rubeai M. G2 cell cycle arrest and apoptosis are induced in Burkitt's lymphoma cells by the anticancer agent oracin. FEBS Lett. 1997 Jan 2;400(1):127-30. Apoptosis, cell growth, cell cycle assessed at 24 to 96 hours.

5. In Results section under subheading “eEF1A2 was highly expressed in HCC liver cancer tissues and HCC cell lines” the way authors have presented range of

expression (values in parentheses following median mRNA levels) is confusing. A small dash (-) in between min and max values will clear this confusion.

Response:

Thanks for this remark. Indeed, it was confusing and the values have been separated by dashes as suggested. Please page 9.

6. Figure 1 legend: SMMC-7721 cells not mentioned in methods or results, not even in the figure? Kindly clarify.

Response:

We apologize for the confusion. SMMC-7721 cells were mistakenly added in lieu of SK-HEP-1. This has been corrected.