Oklahoma State University
Center for Health Sciences,
Department of Biochem & Microbiology,
1111 West 17th Street,
Tulsa, Oklahoma 74107-1898.
Ph. No. 918-561-1231
E-mail: rashmi.kaul10@okstate.edu
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To,
Ze-Mao Gong,
Scientific Editor,
World Journal of Gastroenterology,
Baishideng Publishing Group Inc.

Subject: Resubmission of ESPS Manuscript NO: 34004

Dear Ze-Mao Gong,

Thank you for giving us the opportunity to revise and resubmit our manuscript titled "Estrogen Receptor (ER) expression in chronic Hepatitis C and Hepatocellular Carcinoma pathogenesis". We thank you and the reviewer for providing insightful comments and suggestions that have been incorporated in the revised version of the manuscript. The manuscript has benefited from these changes and this revised version looks much strengthened.

We have responded to all the suggestions of the reviewer and editor and revised the manuscript accordingly. We have listed all the comments by the reviewer below along with our responses and have indicated page numbers of the manuscript where changes have been highlighted by color.

Reviewer concern: "However, the role of ERalpha and beta isoforms was not described or discussed at all; although the authors detected expression of ER isoform as can be visible on the western blots".
Our response: We have added more information to the Discussion section of the manuscript on the role of ERα and ERβ isoforms or variants in different cancers in addition to liver cancer on page 21 of the Discussion section.

Reviewer concern: "Page 9: The info about Abs is insufficient. "...probed with antibodies specific for human ERα (Fitzgerald Industries International, Acton, MA, United States), human ERβ (...)" – authors should specify exactly the region of the receptor that is recognized by the Abs. Are the Abs made in mouse or rabbit? Catalog #? Clone? Etc."
Our response: We thank the reviewer for bringing our attention to the details of the antibodies. As requested by the reviewer, we have included specifications of antibodies in the Materials and methods section in pages 9 and 10 of the manuscript.

Reviewer concern: Page 10: the positive control IHC should be presented (it can be presented as Supplementary material/figure). The best ER positive control samples are breast cancer tissues. Readers need to see how used (novel) Abs react with positive control samples and whether the nuclear and cytoplasmic ER can be detected in breast cancer samples using these Abs.

Our response: We agree with the reviewer about the need for appropriate controls. The antibodies that we have used in our immunohistochemical analyses have been widely used in many studies to detect ER expression in a variety of tissues of human origin in addition to breast cancer tissues. Additionally, for reference purposes, we have provided below examples of some of the studies found in the literature where these antibodies have been utilized for immunohistochemistry in human tissues.

- Selected publications for ERα, clone MC-20, antibody:
    - Human Hypothalamus: Kruijver FP, Balesar R, Espila AM, Unmehopa UA, Swaab DF. Estrogen receptor-alpha distribution in the human hypothalamus in relation to sex and endocrine status. The Journal of Comparative Neurology, 2002, Vol. 454, pp 115–139. PMID: 12412138.
    - Human Heart: Mahmoodzadeh S, Eder S, Nordmeyer J, Ehler E, Huber O, Martus P, Weiske J, Pregla R, Hetzer R, Regitz-Zagrosek V. Estrogen receptor alpha up-regulation and redistribution in human heart failure. FASEB J, 2006; Vol. 20, pp 926-934. PMID: 16675850.
    - Breast cancer: Giulianelli S, Vaqué JP, Soldati R, Wargon V, Vanzulli SI, Martins R, Zeitlin E, Molinolo AA, Helguero LA, Lamb CA, Gutkind JS, Lanari C. Estrogen Receptor Alpha Mediates Progestin-Induced Mammary Tumor Growth by Interacting with Progesterone Receptors at the Cyclin D1/MYC Promoters. Cancer Res, 2012, Vol. 72, 2416-27. PMID: 22396492.

- Selected publications for ERβ, PPG5/10, antibody:
    - Human Bladder cancer: Tan W, Boorjian S, Advani P, Farmer S, Lohse C, Cheville J, Kwon E, Leibovich B. The Estrogen Pathway: Estrogen Receptor-α, Progesterone Receptor, and Estrogen Receptor-β Expression in Radical Cystectomy Urothelial Cell Carcinoma Specimens. Clinical Genitourinary Cancer, 2015, Vol. 13, pp 476-84. PMID: 25981333.
    - Human Breast cancer: Saunders PTK, Millar MR, Williams K, Macpherson S, Bayne C, O'Sullivan C, et al. Expression of oestrogen receptor beta (ERβ1) protein in human breast cancer biopsies. British Journal of Cancer, 2002, Vol. 86, pp 250-6. PMID: 11870515
    - Human Prostate tissue and cancer: Fixemer T, Remberger K, Bonkhoff H. Differential expression of the estrogen receptor beta (ERβ) in human prostate tissue, premalignant changes, and in primary, metastatic, and recurrent prostatic adenocarcinoma. Prostate, 2003, Vol. 54, pp 79-87. PMID: 12497580

Reviewer concern: "Figure 1A. Western blots were overexposed/ bleached. The background level is not visible. The blots look different to those provided in Figure 2 and 3. Why? On Fig2 and 3 the few other ER bands (ER variants) are recognizable, which is normal and happens often with polyclonal Abs. If authors intentionally cut off the upper and lower ER bands, they should replace the figure with larger cuts so it will be possible to see the expression of all ER bands in liver samples. The band size should be also marked. The presence of ERalpha/ beta isoform variants in the liver samples should be described in the results section and in Discussion."

Our response: We are thankful and agree with the reviewer for his suggestion that band sizes should be incorporated in the Western blots. We evaluated the ER expression in whole tissue lysates from 20 normal subjects (table 1). In Figure 1A, we are showing the ER expression in whole tissue lysates of 6 normal subjects while in Fig. 2D we are showing the ER expression in whole tissue lysates of the remaining 14 normal subjects in addition to HCV and HCC subjects. In Figure 3A we are demonstrating the ER expression in nuclear and cytoplasmic extracts in normal, HCV and HCC subjects. Based on the reviewer's recommendations, we have added the band size on all our Western blots. We have also elaborated on ERα and ERβ isoforms in the liver in our discussion section on page 21.

Reviewer concern: "Figure 5D – is too small and it is nearly impossible to see the localization of the protein. That makes the figure useless. It has to be enlarged or insertion images with higher magnification should be provided."
Our response: We agree with the reviewer's concern and have modified Figure 5D based on the reviewer's recommendations to show an enlarged image of the tissue as an inset.

Reviewer concern: "Discussion section: The role of ERalpha/beta isoforms attracts a lot of attention in the area of various intestinal cancers. Authors should make a statement about this as supporting fact for their investigation and add couple of relevant references including these: Xu CY, Guo JL, Jiang ZN, Xie SD, Shen JG, Shen JY, Wang LB. Prognostic role of estrogen receptor alpha and estrogen receptor beta in gastric cancer. Ann Surg Oncol. 2010 Sep;17(9):2503-9. doi: 10.1245/s10434-010-1031-2. ? Sukocheva OA, Wee C, Ansar A, Hussey DJ, Watson DI. Effect of estrogen on growth and apoptosis in esophageal adenocarcinoma cells. Dis Esophagus. 2013;26(6):628-35. doi: 10.1111/dote.12000."
Our response: The reviewer brings up a valid point and we have modified our discussion to include more information on the role of wt ERα and ERβ as well as their variants in gastrointestinal cancers in page 19 and 21.

Reviewer concern: Discussion section page 17: "We also found significant differences in the ERα: ERβ expression ratio in males vs females." Authors should be more specific here and make precisely statement whether the ration is lower or higher in males etc. Page 17: "…there was a significant change in ERα but not ERβ expression only in diseased" – again: what change – increase or decrease? – be specific, avoid misleading generalized statements.
Our response: We have made the relevant modifications to specify the changes or alterations that we observe. These changes have been highlighted in the manuscript.

Reviewer concern: Discussion section: In majority of the relevant sentences the words "change", "difference" etc should be replaced with "increase" or "decrease" according to the observed effects.
Our response: We have made the relevant modifications to specify the changes or alterations that we observe. These changes have been highlighted in the manuscript.

Reviewer concern: The study limitations should be mentioned in the Discussion (small group/ sample size etc).
Our response: We have indicated the limitations of the study in the results (page 15) as well as discussion section (page 21 and page 22).

Reviewer concern: Page 20: "We did not evaluate the expression of the variants due to the lack of commercially available reliable antibodies." It is not entirely true. The sentence should be re-written. The involvement/role of ERalpha/beta isoforms should be presented in more details
Our response: We have taken the reviewer's concern into account and rewritten the statement on page 21 based on the reviewer's recommendation. We would like to emphasize that the present study was focused to fill the gap in knowledge for the expression of wild type ERα and ERβ in normal and diseased livers. We have observed other bands in some of the diseased samples that might correspond to ER variants/isoforms, which need to be confirmed. We have initiated a separate study to investigate the role of variant ERα and ERβ in HCV-mediated pathogenesis. In the current manuscript, we have rewritten the sentence specified by the reviewer as per his/her suggestions. As suggested, we have added more information on the role of ERα and ERβ isoforms or variants in different cancers in addition to liver cancer on page 21 of the Discussion section.

Editor's concerns: The editor highlighted certain parts of the manuscript that needed editing, which includes writing the manuscript no., documentation for the Supporting foundations section, Audio core tip file, modifying the Aim of the abstract and ensuring that it is within 20 words, writing the Comments section and ensuring that the References are not duplicated.
Our response: We thank the editor for bringing all this information to our attention. We have taken care of all of the Editor's concerns in the revised manuscript.

I look forward to working with you for the publication of this manuscript.

Thanks,

Rashmi Kaul

Sincerely,
Rashmi Kaul, Ph.D.