

Dear Editor:

Thank you so much for your letter of Sep. 28, 2016 and the insightful and constructive comments on our manuscript by expert reviewers. We have revised the manuscript according to these comments. We made the following point by point responses to address the reviewers' comments. The changes in the text are highlighted in blue.

Reviewer #1:

Comments to the Author(s):

The manuscript by Bu et al. describes the potent new capacity of melatonin playing the role in treatment of hepatocellular carcinoma patients. In general, enclosed set of data are very interesting nevertheless, major editorial corrections are strongly needed to improve the quality of this report. Small remarks that should be corrected in the text are listed below: 1. Please correct the mistake in the title: There is: Melatonin, a novel selective..... Should be: Melatonin, a novel selective.....

Answer:We have now corrected that.

2. Abstract: Please mention the full names followed by used abbreviations such as UPR, ATF-6, COX-2, CHOP, HCC. Please be consistent to use the same way of abbreviations throughout the whole manuscript. Sometimes it is written e.g. ATF6 and ATF-6 or COX2 and COX-2. Please change it accordingly. a. Line 7: There is:need to be clarified. Should be:need to be clarified. b. Line 12: There is:(an ERinducer).... (missing space) Should be:(an ER inducer)... c. Line 16 and 17: There is: In conclusion, There....selective.... Should be: In conclusion, there....selective....

Answer:We have now corrected that.

3. Introduction: a. Page 2, Line 5: There is:HepG-2.... Should be:HepG2....

Answer: We have now corrected that.

4. Materials and methods: a. Reagents (Line 11 and elsewhere): There is:TRIZOL.... Should be:TRIZol? Reagent.... b. Hepatocellular carcinoma specimens Line 9 and 10: Please be consistent using the numbers pointing out the patients, not sometimes written with numbers and words. c. Immunohistochemical analysis Line 6: Please write in brackets the pH used for PBS. d. Cell culture ? Remove the sentence about the source of HepG2 cell line mentioning the Argentinian boy. It is simply redundant. ? DMEM was high or low glucose. Please specify and write it. Line 4: There is: The cell was cultured.... Should be: Cell culture was carried out in.... Line 7: There is: CO2 atmosphere. Should be: CO2 atmosphere. e. Flow cytometry Line 8: There is: A total of 1*10⁶ cell/mL.... Should be: A total of 1×10⁶ cell/mL.... f. qRT-PCR ? Please describe more precisely the conditions for performed PCR i.e. temperatures and times for denaturation, annealing, elongation etc.

Answer: We have now corrected that.

5. Discussion: Page 3: Together with given citations of #19 and #20 add also essential references as follows: - Kleszczyński K, Zillikens D, Fischer TW. Melatonin enhances mitochondrial ATP synthesis, reduces reactive oxygen species formation, and mediates translocation of the nuclear erythroid 2-related factor 2 resulting in activation of phase-2 antioxidant enzymes (γ -GCS, HO-1, NQO1) in ultraviolet radiation-treated normal human epidermal keratinocytes (NHEK). *J. Pineal Res.* 61 (2016) 187–197. - Kleszczyński K, Tukaj S, Kruse N, et al. Melatonin prevents UVR-induced alterations in plasma membrane potential and intracellular pH in human keratinocytes. *J. Pineal Res.* 54 (2013) 89–99 - Fischer TW, Kleszczyński K, Hardkop LH, et al. Melatonin enhances antioxidative enzyme gene expression (CAT, GPx,

SOD), prevents their UVR-induced depletion and protects against formation of DNA-damage (8-hydroxy-2'-deoxyguanosine) in ex vivo human skin. J. Pineal Res. 54 (2013) 303–312 - Fischer TW, Scholz G, Kn?ll B, et al. Melatonin suppresses reactive oxygen species induced by UV irradiation in leukocytes. J Pineal Res 2004; 37:107–112. - Slominski A, Pisarchik A, Zbytek B, et al. Functional activity of serotonergic and melatonergic systems expressed in the skin. J Cell Physiol 2003; 196:144–153.

Answer: We have now included all the references mentioned.

6. Legends: Please adapt the statistics labeling appropriately. For instance in Fig. 2, there are **, # and ## but it is not consistent with the Legends. Please correct elsewhere accordingly! a. Fig. 2 - Please mention about incubation with TM what is seen in the graph but missing in the legend description. - In Line 3, please add 10-9 mol/L, it is simply missing! Is

Answer: We have now corrected that.

Reviewer #2:

The article “Melatonin, a novel selective...” by L. Bu et al. deals with the mechanism(s) of melatonin-induced apoptosis in human hepatoma cells. Using a few modern methods, immunohistochemical analysis, flow cytometry, TUNEL assay, Western blotting, qRT-PCR, the authors showed a close relationships between the level of the expression of the activating transcription factor 6 (ATF6) and COX2. Using cell line Hep G2, it was shown that melatonin could selectively block ATF 6 and then inhibit COX2 expression, leading to enhanced liver cells apoptosis. These data provide a new mechanism by which melatonin downregulates COX2 expression and induces apoptosis by selectively targeting ATF6 in human HCC cells under endoplasmic reticulum stress. The article will be interesting for readers of this Journal. My minor comments: 1. The abbreviations in the Abstract should be explained.

Answer:We have now added that.

2. It should be interesting to discuss the mechanism(s) of melatonin effect: antioxidant properties, an interaction with nuclear receptors,

Answer:That's a good suggestion. We have made the following discussion.

Melatonin is able to prevent oxidative stress through both its free radical scavenging effect and by directly increasing antioxidant activity^[19-25], and different studies have demonstrated its protective role against oxidative damage induced by drugs, toxins, and different diseases^[26]. In additional, melatonin also acts complex functions through specific nuclear and plasma membrane receptors^[27, 28]. Melatonin MT1 and MT2 receptors are G protein coupled receptors expressed in various parts of the central nervous system and in peripheral organs, which mediate intracellular effects depending on the changes in intracellular cyclic nucleotides (cAMP, cGMP) and calcium levels, activation of certain protein kinase C subtypes, intracellular localization of steroid hormone receptors and regulation of G protein signaling proteins. Alterations in melatonin receptor expression and following abnormal signal pathway as well as changes in endogenous melatonin production contribute to the pathophysiology of various diseases including sleep disorders, depression and Alzheimer's disease^[27, 28].

3. The manuscript should be edited.

Answer:We have now added that.

We believe that thanks to the reviewers' excellent comments we could significantly improve and clarify our manuscript, and trust the revised manuscript will meet your approval.

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