São Paulo, May 12th, 2022

Reviewer's report:

Reviewer 2:

The manuscript "Disruption of redox homeostasis by alterations in nitric oxide synthase activity and tetrahydrobiopterin along with melanoma progression" is a research article regarding the possibility that disruption of cellular redox homeostasis by altered BH4 concentration could be used as a therapeutic strategy for melanoma treatment, depending on the stage of the disease. I appreciate this manuscript that is well written and easy to read, the conclusions are supported by results and the work in general will be interesting for the journal readers. However there are some concerns that authors should address before the manuscript could be considered for publication:

1. **Figure 1: why was GCH1 expression valuated only at mRNA level, while for GTPCH1 authors performed western blot analysis?**

   GCH1 is the gene that encodes the protein GTP-cyclohydrolase I (GTPCH1). So, *GCH1* expression was analyzed by real time PCR and the protein amount by western blot.

2. **In the introduction section authors should provide more details about melanoma impact; indeed, authors correctly state that cutaneous melanoma is the most aggressive type of skin cancer, but they should also underline that cutaneous malignant melanoma represents less than 5% of all malignant skin neoplasms. Furthermore, authors should provide more details about the classification system of melanoma, which differs from other solid tumors (PMID: 34638427).**

   Thank you for the suggestions. The introduction was improved with this information as suggested by the reviewer.

3. **Line 419: "was grown in DMEMF12 at pH 7.2 supplemented with and supplemented." please correct.**

   Thank you for the observation. The text was corrected.

4. **Line 420: "1,4 uM" should be written "1.4". Several mistakes of this type are present in material and methods. Please correct replacing comma with a dot everywhere through the text (e.g. line 433 "0,5" etc…).**

   Thank you for the observation. The text was corrected.

5. **Please provide details about the manufacturer of serum and media.**

This information was included in the manuscript.

6. **Line 443: a dot in "NO analyzer." should be removed.**

   Thank you for the observation. The text was corrected

7. **Line 486: "RNAm Expression Analysis": do authors mean mRNA. Please correct.**

   Thank you for the observation. The text was corrected.

8. **Line 488: Please provide details about cDNA synthesis and primers used for the Real-Time PCR.**

   The information was included in the material and methods section of the manuscript.

9. **Line 491: "2-ΔΔCq method": -ΔΔCq must be written in upperscript.**

   Thank you for the observation. The text was corrected.

10. **Line 507: please provide details about blocking and manufacturer and dilution of the secondary antibody.**

    The information was included in the text.

11. **In the discussion section (line 335) authors underline that all melanoma cell lines showed higher O2•- levels and lower NO levels compared to melanocytes. This is a very important result and authors should underline that these results are in accordance with what is reported in literature, since in comparison with other solid tumors, ROS are particularly elevated in melanomas (PMID: 35453297). Indeed, increasing ROS production seems to be a promising therapeutic strategy for enhancing chemotherapy efficiency in melanomas (PMID: 33297311). Please integrate the paragraph with these considerations.**

    We really appreciate the suggestions and the information were included in the discussion section of the manuscript.

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