Author's response to reviews

Title: Absence of Pathogenic Mitochondrial DNA Mutations in Mouse Brain Tumors

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Author's response to reviews: see over
Dear BioMed Central Editorial Team,

After reviewing the comments made by the reviewers, the following major changes were made to the manuscript to address the criticisms. One of the reviewers, Carlos Sonnenschein, encouraged us to mention a brief alternative explanation for the development of our brain tumors. We address this suggestion with a new paragraph (Paragraph 3 – pg.11) in the discussion:

Most tumors including brain tumors express abnormalities in the number and function of their mitochondria [60, 61]. Warburg originally emphasized that the high glycolytic rate of tumors results from diminished or disturbed respiration [24, 25]. Later studies in a variety of neural and non-neural tumor systems showed that these respiratory disturbances could involve abnormalities in TCA cycle components, alterations in electron transport, and deficiencies in oxidative phosphorylation [62-66]. Recent studies also suggest that mtDNA mutations may contribute to the respiratory defects in cancer [18]. Our findings indicate that diminished respiration and the glycolytic dependence of the chemically induced CT-2A and EPEN brain tumors or the two spontaneous VM brain tumors studied here do not result from somatic mutations in their mtDNA.

Two sentences in paragraph 2 (pg.3-4) of the introduction were also added to address this criticism:

Otto Warburg originally emphasized that the high glycolytic rate of tumors resulted from diminished or disturbed respiration [24, 25]. Recent studies suggest that mtDNA mutations may contribute to the respiratory defects in cancer [18].

After reviewing the suggestion to change the title of the manuscript to “The Search for Pathogenic Mitochondrial DNA Mutations in Mouse Brain Tumors”, we have decided to stay with the title “The Absence of Pathogenic Mitochondrial DNA Mutations in Mouse Brain Tumors”. Since, this was only a suggestion and not a requirement, we feel that the original title would fit the paper better.

In regards to comments made by second reviewer, Jun-Ichi Hayashi, we have decided to address the criticism in the following manner. With respect to our conclusion that mtDNA mutations do not likely contribute to the initiation or progression of mouse tumors, we would like to emphasize that in regards to the number of tumors examined, we make our conclusion based on the data for these brain tumors (Abstract: Conclusion) and not on all tumors, since the sample size is limited. In addition, we stated that “Although we did not find any mtDNA pathogenic mutations in these brain tumors, we do not rule out the possibility that such mutations might occur in other spontaneous mouse brain tumors or in mouse brain tumors induced with other chemical carcinogens” (Discussion: Paragraph 2, pg.12). Also, since all of the tumors examined are of different cell origin and are spontaneous as well as chemically induced, we feel that these factors provide a broad enough spectrum for our conclusion about the nature of mtDNA mutations in these tumors.
Relative to the second discretionary revision suggested by the second reviewer, we agree with the statement that the topic of homoplasmic mtDNA mutations is a controversial subject matter. Since, the mutation was lost in later independent isolates of the cell line (Paragraph 3, pg.9), we feel that this might be the result of a different type of selective process relative to Polyak et al. 1998. Also, since these are tumor cell lines derived from the most aggressive malignant cells of the original tumor (paragraph 1, pg.11), these would allow for a different interpretation of selective pressure relative to mitochondrial homoplasmy, which might complicate the discussion.

Other minor changes were made to the manuscript include adding and clarifying strain designations in the methods section of the abstract. Also, wording was changed in the conclusion section of the abstract for clarity. All reference associated with the VM spontaneous tumors VM-NM1 and VM-M3 were changed to VM-NM and VM-M, respectively for ease of association in this paper. This included all changes in the text of the paper and in Table 3 and Figure 1. Also, Accession numbers for the GenBank sequences corresponding to the C57BL/6J, VM, and their corresponding syngeneic tumors were added to Table 2. The accession numbers are:

DQ106412 (for C57BL/6J, CT-2A, and EPEN)
DQ106413 (for the VM strain, VM-NM, and VM-M)

A few other very minor grammatical changes were made where a period was added as well as a few words were changed for clarity. If there are any questions regarding these changes, feel free to e-mail me at Kiebish@bc.edu regarding manuscript 8122691007001700.

Regards,
Michael Kiebish