Reviewer's report

Title: Effect of rotary magnetic fields on melanoma: tumor inhibition and immune activation

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Reviewer: Robert M Lafrenie

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The manuscript, "Effect of rotary magnetic fields on melanoma: tumor inhibition and immune activation", describes in vitro and in vivo experiments that show exposure to a particular rotating magnetic field pattern (0.4T, 7.5 Hz) slows cancer cell growth. The in vitro data shows that treatment of B16-F10 cells with the rotating field causes a small increase in annexin V staining cells, a small shift in cell cycle, and a small decrease in cell proliferation (CFSE dilution and CCK-8 staining). In vivo, mice were transplanted with B16-F10 cells and exposed to the rotating field. EMF-exposed mice were shown to survive longer (although p=0.33), show lower levels of Ki-67 proliferation marker, enhanced anti-tumor IP-10 cytokine and decreased chemokines and cytokines, decreased number of splenic Tregs and increased dendritic cells. In my opinion, additional experiments need to be performed in order to support the conclusions made regarding the relatively small differences in data.

1. The apparatus for generating the 0.4 T, 7.5 Hz needs to be more fully described. The exposure volume for the cells and animals needs to be described. The characteristics of the magnetic field are critical for understanding any biological effects and therefore a clear and reproducible description of the apparatus and field exposures are required.

2. The effects described are quite small with the changes in proliferation being 15-30% after 5 days (CCK-8 counting and CPSE dilution). These should be supported by other methods such as simple cell counting.

3. The data in figure 1 is not very compelling. The changes in flow cytometry show changes from 15% to 21% for annexin staining. In my experience, inter-experiment variation is frequently at least this variable, and the error bars (SEM) shown are much smaller than I usually get for this experiment. Similarly, the cell cycle variability seems to show unexpected lack of variability (SEM). I would also like to know how the samples were gated for the flow cytometry since no sub-G1 cells are seen which seems inconsistent with the relatively large number (15-20%) of annexin stained apoptotic cells. In order to be convincing, additional experiments should be performed to verify this data. For example, caspase-3 cleavage, DNA fragmentation gels, or nuclear morphology, should support the annexin V staining, and cyclin measures, or BrdU labelling should support the proliferation data.
4. Figure 3 (electron microscopy) needs to be supported by determining the number of affected nuclei in each condition to determine if this effect is significant.

5. For the in vivo experiments, the statistics underlying the survival curves needs to be better explained. IF this is a Kaplan-Meier statistic, then p=0.33 is not significant and therefore the magnetic field has no effect.

6. The relative number of Ki-67 staining cells for each condition also needs to be counted for each condition. What is being sectioned for Ki-67 staining in the mice without tumours?

7. The levels of plasma cytokines and splenic T cells seem reasonable, but it would be nice to see the levels of immune mediators and cells in the tumor itself. Are these cells sequestered in the tumor?

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

No competing interests.