

Review of: "Metadichol Induces CD14 Glycoprotein Expression in Human Embryonic Stem Cells and Fibroblasts"

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Potential competing interests: No potential competing interests to declare.

The authors address a fascinating topic of immune system modulation for the treatment of chronic pathological conditions using innovative substances. However, the work requires several revisions, without which it should not be published.

Major Revisions:

- 1. Authors must declare the origin of the human embryonic stem cells. At this time, this is not clear.
- 2. The results section needs improvement. The data needs to be described carefully and separately. Although the trend between rt-pcr and western blot is similar, there is no strict correspondence between the increase in messenger and the amount of encoded protein. This evidence needs to be described and commented on.
- 3. There is no statistical analysis in the experimental data nor mention of replicates. The authors can perform an ANOVA test using statistical tools, such as GraphPad, to demonstrate the accuracy and reliability of their data.
- 4. Authors should show the toxicity of metadichol at different concentrations. A cell viability test should be reported as a treatment effect in the cell lines used.
- 5. The discussion needs to be improved. In this section, the results of the current work and its limitations should be commented on. Previous works should only be cited. For this, Table 2 should be eliminated.

Minor Revisions:

- "The search results" sentence is unclear and ambiguous. If it refers to literature data, it would be more correct to replace it with previous studies or similar.
- 2. It is not necessary to describe the entire protocol of a commercial reagent widely used in the world, such as TRIzol.
- 3. In rt-PCR experiments, authors should report the amount of cDNA used and not the volume of 1 µL.
- 4. No reference in the text to Table 1 or to which antibodies were used for the western blots. The authors can report in a table the primary and secondary antibodies used in the experiments and their species of origin.
- 5. Figures could be improved:
 - 1. In Table 1, the housekeeping gene is reported as GAPDH, while in Figure 1, it is actin. Which housekeeping gene is correct?
 - 2. In Figure 3, there is mention of NHDF cells, but there is no reference in the text.



- 3. Tables of Figure 1 and Figure 3 should be standardized. The graphs could be unified as separate panels of the same image.
- 4. The western blot results for NHDF cells are not shown.