Review of: "Study Importance of Mutation in RAD50 Gene Based on in Silico Analysis of in Vivo Mutation"

Noria Bouras

Potential competing interests: No potential competing interests to declare.

Hello. Here is my expert report.

1. Why was this specific protein (RAD50) chosen for the study?

2. What are the reasons behind the selection of this particular mutation and its specific location? Are there other similar studies that have examined this mutation or a similar one?

3. Why was the zebrafish chosen as a model for this study?

4. What are the reasons for the reduction in the number of viable embryos, which did not exceed 72 hours of development?

5. The CRISPR/Cas9 approach was not described in detail or emphasized in the study, particularly concerning the deletion and insertion procedures.

6. What types of controls were used to confirm the validity of the obtained results?

7. Regarding the discussion on the in silico study:

- The physicochemical properties of both aspartic acid and asparagine are missing to guide the discussion based on their properties.
- Is the region where the mutation would occur conserved or not? This could predict whether the residue in question plays an important role in the maintenance and function of the RAD50 protein.
- The amino acids that were linked to residue 641 by hydrogen bonds have been described, but are they really amino acids of the RAD50 protein? Could they be other interactions with amino acids of the MR11 subunit or the Nbs1 structure?
- Furthermore, it would have been desirable to study, via the in silico study, the interactions with these two structures that form the MRN complex and/or with the ATR protein to predict the effect of the mutation on the stability of the complex, considering the assemblies of different proteins that are highly likely to be biologically relevant and can form complexes with the RAD50 protein.
- Review the representation of Figure 7 to improve its clarity.
- Investigate whether the sites of amino acids prone to mutations into asparagine are located within catalytic domains or involved in interactions with other proteins. Which codons are they coded by? And on which exon of the gene?

8. Lastly, the perspective section is missing, explaining why and for what purpose this work was carried out. This should include the motivations behind the study, its potential implications for future research, and its impact on areas such as gene therapy.