Reviewer's report

Title: Glibenclamide inhibits cell growth by inducing G0/G1 arrest in the human breast cancer cell line MDA-MB-231.

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Reviewer number: 1

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The authors report a biochemical characterization of the actions of 25 micromolar glibenclamide in the MDA-MB-231 ER-negative human breast tumor cell line. The purpose of the work is clarification of potential mechanisms of the anti-tumor actions of glibenclamide which were previously observed in the NMU mammary tumor rodent model. The authors also seek to identify potential mechanisms by which co-administration of tamoxifen enhanced the anti-tumor effects of glibenclamide which was reported in the NMU mammary tumor model.

The experimental work (methods and results) is clearly described and appropriate targets including cell cycle proteins, cellular senescence and apoptosis were included. The experimental data are sound and are evaluated by appropriate statistical methods. Some new information is reported including: (1) reversible cytostatic actions of glibenclamide in MDA-MB-231, (2) G1 cell cycle accumulation, (3) lack of cellular differentiation as measured by Nile Red lipid assay, (4) low level of senescence induction, (5) limited involvement of apoptosis in the glibenclamide response.

Major Compulsory Revisions:

1. The work suffers from a lack of experimental support for a clearly defined mechanism to explain the anti-proliferative activity of glibenclamide. The authors refer to previous work supporting potassium channel involvement. However, there is no experimental work in this paper that tests the potassium channel concept. There is mention of the use of minoxidil, but this data is not presented. This would be important data to include. Furthermore, a direct test for the presence of KATP channels in MDA-MB-231 cells and demonstration of the concentration response to glibenclamide would strengthen this work.

2. The mechanism of anti-proliferative activity of tamoxifen in the MDA-MB-231 cell line needs to be discussed in this paper. MDA-MB-231 cells are estrogen-receptor negative and the actions of tamoxifen in this cell line might be mediated through tamoxifen’s activity as a protein kinase inhibitor rather than as a SERM. Nevertheless, tamoxifen did not augment the actions of glibenclamide; the tamoxifen data distracts from the central focus on glibenclamide.

Minor Revisions:

1. Check the spelling of senescence.
2. The use of sodium butyrate as the positive control for the Nile Red assay requires some explanation.

Discretionary Revisions
1. Omission of the tamoxifen concentration – response data and the combination of tamoxifen + glibenclamide data.

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

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.