Reviewer’s report

Title: Limitations in high-throughput drug screening on a cellular model for Friedreich ataxia

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Reviewer: Brigitte Sturm

Reviewer’s report:

In this manuscript the authors describe a high-throughput drug screening in a cellular murine fibroblast model with strongly reduced levels of frataxin. For the screening the Prestwich Chemical Library was used. Cell proliferation was assessed 72 hours after cell treatment with 25µM of each compound dissolved in DMSO.

The authors found 87 hits (out of 1120) during the first screening with 25µM of each compound. Those 87 hits were further evaluated with more concentrations but at the end they could not identify any reproducible hit at all.

Performing a high throughput screening for FRDA is urgently needed and highly appreciated.

Although no positive reproducible hit was found this paper may contribute positively to the development of further screenings and perhaps some lessons can be learned from these data presentations.

Major:

1) The main criticism arises from the cell line which was used in this project. The authors based the HTS on measuring ATP levels as a parameter for cell proliferation. They also state that there is a lack of measurable catalytic function for frataxin.

Using a parameter which is only present in artificial genetically modified systems seems not useful for identifying chemical compounds which could possibly improve the quality of life of FRDA patients. As far as I know there are no data published which show decreased cell proliferation in FRDA patient samples. This should be discussed in more detail.

2) The paper seems more like a presentation of a cell line rather than the focus on the limitations of HTS in FRDA. The title of the manuscript is not sound with the content of the paper.

3) The paper can be shortened by focusing on the used clone R2C1. (Figure 1, 2).
4) It is not clear which clone was used for ISC protein activities?

5) It is not clear if the authors have thawed fresh cell aliquots regularly or if they really used the same cells for more than 2 years as mentioned in the discussion. This should be explained!

6) As shown in Figure 4 C there are a high number of hits decreasing ATP production in both clones. The meaning of this is not mentioned and should be discussed. It would be of interest learning more about drugs which decrease ATP production in frataxin deficient clones but not in controls. Maybe they could also indicate some mechanism of the physiological function of frataxin.

7) The average luminescence rate of % of control as shown in Figure 4 is approx. 100% in R2m cells and approx. 110% in R2C1. This should be at least discussed.

8) Statistical analysis of Figure 5 is missing. It is not clear whether the Mthfd2 levels are from clone R2C1 at 16 % frataxin mRNA levels or 29 % mRNA levels? It is not clear why R2m with 50 % frataxin levels (Figure 5A) was used as control. Wild type cells should be used for this experiment.

The necessity of this graph is questionable for the importance of the message of the paper.

Minor:

Methods:

1) Biochemical analysis of respiratory chain enzyme complexes should be described instead of cited from other papers

2) Incubation media should be defined for HTS.

3) The sedimentation time of 30 minutes after seeding seems very short considering the cellular stress after trypsinisation

4) It is not clear what the variation of cell number in the wells using Biomek 2000 is.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

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

Declaration of competing interests:

I declare that I have no competing interests