Reviewer's report

Title: Human Sulfatase 2 inhibits in vivo tumor growth of MDA-MB-231 in human breast cancer xenografts

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Reviewer: Wen Jiang

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The study by Petersen and colleagues have shown that breast cancer cell line MDA MB 231, when transfected with sulfatase-2 (and in combination with sulfatase-1), had a reduced rate of growth in vitro and in vivo when injected in nude mice. The in vitro effect co-incided with a reduction in the phosphorylation of ERK. However, intratumour injection of recombinant sulfatase-2 had no effect on the growth of breast tumours. Certain aspects of the study are interesting. However, there are major concerns with the study.

Main concerns:

1. Given the inconsistency of the results: i.e. in vivo tumour growth rate in models injected with sulfatase-2 transfected cells and injected with recombinant sulfatase-2, one would anticipate that more than one cell lines are to be tested in order to consolidate a valid argument and that refined strategies were attempted (also refer to points 3 and 4).

2. Figure-1C and D: recombinant hSulf2 was said to have a relatively low stability, <2 days. In fact, more than 50% of the activity seemed to be lost with 5 hours. It is not clear how this reflected in the in vitro and in vivo models.

3. Figure-2 showed an inhibitory effect of sulfatases on the growth of the cells by an MTT method. It will be useful to demonstrate the nature of the inhibition: change of cell cycle, apoptosis or necrosis. Given the limited data from the study, this will be a desirable and welcoming addition.

4. Figure-3. Although ERK phosphorylation was shown to be inhibited by hSulf2, it will be necessary to show if the phosphorylation of receptors for FGF and EGF, two cytokines used in the study, were also affected, to establish a context. In addition, phospho-ERK in the figure are seen as double bands. This needs further clarification.

5. In vivo delivery of rSulf2 resulted in no change in tumour growth. Although the possibility of continued availability of the protein from tumour cells was discussed, critical factors that low stability and bioavailability should be discussed and alternative strategies could have been used in answer these questions. Why no dose response was tested and why systemic injection was not evaluated, given that intratumour injection is so much unlikely to happen in a clinical setting? It is not clear how rSulf2 was formulated, in medium or in serum. In either case, why was the treatment only lasted for 5 days and not longer? Given the said short life of rSulf2, why a method allowing persistent delivery was not used, for
example semipermeable minipump?

6. Conclusion is not supported by the data. It is concluded that 'in vivo progression of human breast cancer xenografts can be inhibited with sulfatase treatment'. In fact, the study showed that intratumour injection of sulfatase-2 had no effect on tumour growth.

**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 have no conflict of interest.