Reviewer's report

Title: Evaluation of novel N-acetylalaninate prodrugs that selectively induce apoptosis in prostate cancer cells

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Reviewer: Ana Bela B Sarmento-Ribeiro

Reviewer's report:

In this paper, Christopher A McGoldrick et al., investigated the effectiveness of four novel prodrug esters: the R- and S- chiral esters of 4-[(nitrooxy)methyl]phenyl N-acetylalaninate (R- and S-NPAA) and the R- and S- chiral esters of 4-[(nitrooxy)methyl]naphth-1-yl N-acetylalaninate (R and S-NQM), in tumorigenic and non-tumorigenic prostate cell lines as well as in COS-7 cells overexpressing human oxidized protein hydrolase, OPH (COS-7-OPH). Their results show that the prodrugs were activated by OPH and subsequently depleted GSH. They also found that the S chiral ester of NPAA (S-NPAA) was two-fold more effective than the R-chiral ester (R-NPAA) in depleting GSH, increasing oxidative stress, inducing apoptosis, and decreasing cell viability in tumorigenic prostate LNCaP cells but had little effect on non-tumorigenic RWPE-1 cells. In addition, they found that S-NPAA induced apoptosis and decreased cell viability in tumorigenic DU145 and PC3 prostate cell lines.

On account of their results authors said that “prostate tumors overexpressing OPH and/or exhibiting a high level of intrinsic oxidative stress may be susceptible to QM generating prodrug esters that are targeted to OPH with little effect on non-tumorigenic prostate cells”.

The question posed by the authors is well defined and the methods appropriate. However, authors should clarify why the studies are not made in all cell lines and why they choose some cell types to do some of the studies and not others. Furthermore, they could explain besides the OPH levels, if other mechanisms could contribute to results observed (ex. Gene mutations, basal antioxidant levels...?)

Major comments:

1. Despite the relevance of the assays, the authors must clarify and justify the differences obtained between cell lines and why the studies are not done in all.
2. They should explain, besides the OPH levels, if other mechanisms could contribute to results observed (ex. Gene mutations, basal antioxidant levels...?)

Other specific comments:

1. The contribution of AKT activation is only a hypothesis, it is not proved in the article.
2. The authors must be more carefully when they said that the prodrugs tested “induced selectively apoptosis in prostate cancer cells”, because they use an in vitro study with cells lines and not primary cultures or animal models.

3. Figs 4, 5 B and C, 6 A and B and 8-B an Table 1 (Fig 10) should be increased in size to facilitate the visualization of results.

4. In the figure legends the acronyms/abbreviations are not spelled which difficult the interpretation.

**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.

**Declaration of competing interests:**

I declare that I have no competing interests' below.