Reviewer's report

Title: Estrogen receptor alpha and aryl hydrocarbon receptor independent growth inhibitory effects of aminoflavone in breast cancer cells

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Reviewer: anna maria musti

Reviewer's report:

The manuscript by Brinkmann et al has examined the effect of aminoflavone (AF) and hydrocarbon receptor (AhR) signaling on cell viability of two triple negative breast cancer (TNBC) cell lines, MDA-MB-468 and Cal51. The authors show that both cell lines are sensitive to AF, although the AhR signaling pathway seems to be responsive only in MDA-MB-468 cells. Furthermore, the authors show that the knock-down of AhR expression was unable to confer AF resistance to both cell lines, suggesting that alternative pathways mediate the effect of AF on cell viability. Cell cycle analysis showed that AF induced accumulation of MDA-MB-468 cells in G2/M, whereas in Cal51 cells it caused transient accumulation of cells in the S phase and induced the senescence-associated marker B-galactosidase. Furthermore, based on the analysis of #H2AX phosphorylation and PARP cleavage, the author suggest that in MDA-MB-468 cells AF-induced DNA damage was rapidly repaired, but longer AF exposure finally induced cell death. In contrast, in Cal51 cells, AF-induced DNA damage remained elevated and ultimately cells entered senescence. Based on their results, the authors conclude that AF inhibits cell growth of two TNBC cell lines in a AhR-independent manner and by inducing cell type-specific DNA damage cellular responses.

Major compulsory revisions:

The experimental quality of this study is very good and all technical methodologies that were used are very appropriate for assessing the author's questions. However, we believe that more experiments are required for turning this study in a novel contribution to the elucidation of mechanisms underlying AF antitumoral action. Previous studies have shown that AF-mediated DNA damage is strictly dependent on AhR-mediated activation of cytochrome P450 enzymes, therefore the authors need to assess the possible mechanism mediating the AhR-independent effect of AF on cell growth. First of all, they have to assess whether the cellular response to DNA damage is the major mechanism underlying the effect of AF in MDA-MB-468shAhR and Cal51shAhR cells. We suggest to assess:

1. the kinetic of #H2AX phosphorylation in response to AF in +/- Dox treated cells
2. the effect of inhibitors of the DNA damage pathway in response to AF in +/- Dox treated cells
3. the potential role of the high basal levels of Sult1A1 expression in the AF effects: we suggest to knockdown Sult1A1 expression in both MDA-MB-468 and Ca51 cells and analyse cell growth and the kinetic of #H2AX phosphorylation.

Minor revisions:

1. The authors should discuss in detail the effect of AhR knockdown in MDA-MB-468shAhR cells (Fig. 4B), as it seems to increase the sensitivity to AF.
2. Duration time of AF treatments shown in fig. 1 and fig.4 have to be written both in the figure legends and methods.
3. The method used for determination of mRNA levels shown in fig.2 has to be written in the figure legend.
4. Previous studies analysing the effect of AF on the DNA damage pathway in MDA-MB-468 have be included in the introduction (for example, Mclean et al 2008) and in the discussion.

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 no competing interests