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: Filippo Acconcia

Reviewer's report:

In this manuscript the Authors describe a potential novel pathway for AF anti-tumor activity in breast cancer cells independent of AhR and ERalpha.

Although the manuscript is interesting and the quality of the data presented are opportune, I have a problem with the main conclusion of the paper.

The main point is that because AhR is located outside of the nucleus the effect observed could be due at least to some extent to the activation of some AF-mediated AhR-dependent extranuclear effects in the chosen cell lines. From this, it derives that the authors are requested to demonstrate:

1-AF binding to AhR
2-ERalpha absence in the cell used
3-Possible AF-dependent extranuclear signalling to apoptosis and senescence.

Detailed suggestions:

In order to demonstrate that ERalpha and AhR are not required for AF action, additional controls are necessary:

Figure 1:

The fact that MDA-MB-231 is insensitive to AF is not correct. Indeed these cells require an higher dose of AF to die.

A panel confirming ERalpha presence and AhR presence in all cell lines should be added. Because ERalpha has also been found in ERalpha-negative breast cancer (Please see the work of Dr Muriel Le Romancer EMBO Mol Medicine performed using proximity ligation assay), both WB and RT-PCR should be performed.

Figure 2.

I believe that the data do not support the conclusions. Indeed, the Authors should demonstrate that AF is binding to AhR both in vitro and, moreover, in the AhR receptor expressed in the cell lines under investigation. This is critical to support the notion that in these cell lines AhR is not required.

Figure 4.
The authors knocked down the AhR receptor and observed that upon AF treatment one cell lines acquire additional sensitivity to this compound and another one is unchanged. Although the explanation that residual effects of the AhR receptor after knock-down makes sense, the immediate conclusion is that AhR does not mediate the effect of AF in these cell lines. However, extranuclear AhR could also play a role.

Additionally, is it possible that if AF does not bind to AhR some mutated form of AhR exist in these cell lines?

Figure 5.

In order to firmly exclude that AhR is not required for AF-induced apoptosis and senescence, all the presented experiments should be repeated in the presence of AhR knock-down.

Because AhR is also located outside of the nucleus and in this cell lines the nuclear effects of this receptor are negligible, I strongly suggest to look to what happens to the AhR located outside of the nucleus. Indeed, if AF binds to AhR in these cell lines it remains possible that some extra-nuclear mechanisms is going on to explain the observed effects. I would test SAPK as JNK or p38 to see if AF modulates such signalling kinases through AhR in order to control apoptosis and senescence.

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.