Reviewer's report

Title: Synergistic action of N-palmitoylethanolamine and the FAAH inhibitor URB597 on melanoma growth

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Reviewer: Guillermo Velasco

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In this manuscript Hamtiaux et al use B16 melanoma cell lines and tumor xenografts generated with these cells to analyze the potential anti-melanoma effect of palmitoylethanolamine (PEA) in combination with inhibitors of the degradation of N-acylethanolamines. One of the weaknesses of this work is the lack of mechanistic explanation for the effect of PEA on melanoma cells and tumors. Administration of PEA or of the fatty acid amido hydrolase (FAAH) inhibitor URB597 alone does not reduce the growth of melanoma xenografts suggesting that increasing the endocannabinoid tone would not be enough as for reducing tumor growth. Likewise, only the combination of PEA and URB597 produces a slight reduction in the growth of melanoma xenografts although the mechanism underlying this effect remains to be clarified.

Major Compulsory Revisions

1. The correlation between pharmacological inhibition of FAAH and MAGL and levels of PEA and the other acylethanolamines needs to be strengthened. Levels of PEA, AEA and 2-AG should be determined in the absence and the presence of – at least – some of the other inhibitors of FAAH and MAGL, so a correlation between levels of each these lipids and the observed decrease in cell viability can be established. Likewise, these results should be confirmed (levels of acylethanoamines and viability should be determined) in melanoma cells treated with PEA and other acylethanolamines upon selective silencing of FAAH and MAGL using for example shRNA or siRNA.

2. The mechanism of PEA-induced cell death is unclear. Selective silencing of CB1, CB2, GPR55, TRPV1, PPAR# and PPAR# should be undertaken in order to completely rule out the participation of any of these receptors on the reduction of cell viability observed in vitro.

3. The combined administration of PEA and URB597 enhances apoptosis in vitro (Fig 3A) but not in vivo. In order to confirm these results, an additional method to measure apoptosis (for example DNA fragmentation and active-caspase 3 immununostaining in vitro and active caspase 3 in vivo) should be used. Likewise, changes in the number, size and distribution of vessels should be analyzed in these tumors (for examples using CD31 immuno-staining) to confirm whether angiogenesis is affected by the treatment with PEA and URB597.

4. The authors perform all the study using a single type of mouse melanoma cell
line (B16). At least a few crucial experiments should be also performed with at least one additional human melanoma cell line.

**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