Reviewer's report

Title: Intratumor cholesteryl ester accumulation is associated with human breast cancer proliferation and dedifferentiation: a molecular and clinicopathological study

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Reviewer: Rafat Siddiqui

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The manuscript by de Gonzalo-Calvo presents data to test the hypothesis that intratumor cholesterol ester (CE) levels are associated with clinopathological variables. Cholesterol ester levels were quantified in Luminal A (ER+/PR+/Her-2-), Her-2, and triple negative tumors (TN). Data demonstrated that breast tumors with the highest CEs were exclusively composed of Her-2 and TN carcinomas, and all tumors in the highest tertile of CE content were grade III. This study concludes that intratumor CE accumulation can be a potential indicator of proliferation and differentiation in human breast cancer. The manuscript is well written; however, I have the following comments:

1. This study used 3 representative classes of breast cancer (Luminal A (ER+/PR+/Her-2-), Her-2, and triple negative) but provided no explanation for why triple positive breast cancers (ER+/PR+/Her-2+) were not included in the present study. Interestingly, few studies have shown that 27-hydroxycholesterol (27HC) mimics estrogen and that it has been implicated in the proliferation and metastasis of ER+ tumors. Because this study found that tumors with the highest CE levels were exclusively composed of Her-2 and TN carcinomas, the authors need to elaborate on the mechanism that explains how high CE is associated with extensive cell proliferation in ER-negative tumors.

2. The concluding figure (Figure 5) indicates that intratumor CE accumulation is associated with aggressiveness, but the study (lines 305-308) could not detect a relationship between intratumor CE accumulation and migration and invasion. As Grade III breast cancers are regarded to be invasive, it is not entirely clear if high CE content in breast tumors with “aggressive” behavior mean extensive proliferation without the ability to invade. This point will need to be clarified for the readers.

3. This manuscript, at numerous places (lines 246, 258, 311, and 320), stated that “intratumor CE accumulation is a good indicator of breast cancer proliferation and dedifferentiation.” This study has used Ki-67 staining for measuring cell proliferation index but marker for differentiation (specific cytokeratins) was not assessed. These statements need to be corrected.

4. Lines 55 - 57: The statement that “Plasma level of …are frequently altered in patients with breast cancer” requires some clarification as to the nature of the
alteration in these patients (are the levels of those metabolites high or low?).

5. Line 62: I am not sure about the term “Plasmatic lipoporteins...”; do the authors mean plasma lipoproteins...

6. Line 81- 82: The statement is confusing. What is the relationship between the data in Table 1 and Spain.

7. The statement presented on lines 103 -104 in the Materials and Methods section is repeated on lines 109 - 110, 135 - 136, and 151 – 152. There is no need to repeat this statement in the Results section.

8. The statement on lines 189 -190 needs clarification. How is high CE associated with the cytoplasmic vacuoles in Her-2 and TN breast tumors? Why are data for luminal A tumors not presented in Figure 1.

9. Lines 207 – 208: It is true that tumors with higher CE tertile results were exclusively composed of Her-2 and TN tumors, but a greater number of Her-2 tumors (6 out of 10) had low CE levels. Similarly it is also true that all tumors with high CE levels were grade III (10 out of 30 total tumors), but a similar number of tumors (10 out of total 30) also had low CE. Calculating the percentage using the number of tumors in a subgroup as compared to the total number of tumors is overestimating the % difference in data reported in Table 4. These points need clarification.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

None