Reviewer’s report

Title: Serum estradiol levels associated with specific gene expression patterns in normal breast tissue and in breast carcinomas

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Reviewer: Krystyna Frenkel

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In this manuscript, the Authors describe their findings that relate estradiol levels in serum to the levels of six genes, which they found to be differentially expressed depending on serum estradiol levels. In the multivariate model, two of the six genes were up-regulated [SCGB3A1 (HIN1) and TLN2], while PTGS1 (COX1) was down-regulated in breast samples obtained from women with high serum estradiol when compared to low serum estradiol subjects. None of the six differentially expressed genes were significantly associated with mammographic density, while estradiol was. Two other genes (GREB1 & AREG) expressed in breast carcinomas were associated with serum estradiol in all cancer patients tested, especially, in ER-# positive cases. In the Discussion section, the Authors relate their results to biological properties of the affected genes, such as tumor suppressor for SCGB3A1 and aromatase stimulator for PTGS1, which induces prostaglandin E2 (PGE2) generation that stimulates tissue aromatase expression, thus increasing estradiol production within the target organ.

An important finding resulting from this study is a relationship between serum estradiol and the differentially expressed genes in normal breast tissue in humans and comparisons to normally appearing tissue in breast cancer patients as well as with cancer tissues. Data were obtained on the total 79 healthy women and 64 breast cancer patients, some of which were used in prior studies where subjects were treated or not with the externally provided estradiol.

Overall, it is an interesting manuscript addressing issues important to the breast cancer field. There are some Minor Essential Revisions and Discretionary Revisions that need to be implemented. For example:

Abstract, paragraph 1: It is stated that “High serum levels of estradiol are associated with increased risk of breast cancer.” However, such risk is age-dependent and is evident especially in post-menopausal women, not all women. The Authors are aware of this, since they correctly stated it in the Background section. Nonetheless, Abstract should contain only correct statements, which easily could be obtained by adding ‘post-menopausal’ before ‘breast cancer’.

Background: throughout this section, it would be useful to provide full names of the presented genes, especially since some of them have more than one abbreviation and not all of them are commonly known to a general reader.
Methods, Subjects & Serum hormone analysis (supplemental Table S1): This S1 Table describes criteria used to determine menopausal status. A question arises as to why in sample #10, subjects that show estimated estradiol of <0.1 are considered pre-menopausal. This low serum estradiol could be present in young women after ovary removal, but if that is so, such subjects should be removed from further evaluation and not be considered ‘pre-menopausal’, since it could be misleading and might skew the results.

Results, last two paragraphs of this section & Table S2: The way the comparisons are presented is confusing. It would be better, if comparisons were always expressed uni-directionally from abnormal to more normal, as for example ‘up-regulated or down-regulated in breast carcinomas when compared with the normal tissue’ or ‘up- or down-regulated in high serum estradiol in comparison to low serum estradiol’ subjects. In Table S2, footnote 2: In the table’s title, it should be ‘carcinomas’ (not “carciomas”). The footnote 2 states “Q-value for genes up-regulated in samples …”, but the first gene listed (AREG) is down-regulated by 52.9% in tumors when compared to normal, while others are up-regulated by 28.4-75.3%. Further, it is not clear why TFF3 is listed twice with different values – is it not the same gene? If there are differences between the two, as they appear to be, some explanation is in order.

Discussion: Since COX2 is usually credited with PGE2 formation and tumorigenic outcomes, it is curious that Authors did not discuss why in their study it is COX1 that contributes to carcinogenesis rather than COX2.

“”, Gene expression in breast carcinomas … (last paragraph): Add ‘of’—“This corresponds well with the interpretation of our findings in normal breast tissue …”

“”, Overall strengths and limitations: space is needed before citing references (49-51).

Reference list: Ref. #2 needs to be corrected.

Table 1: It would be better if in ‘B’ all comparisons go in the same direction, i.e., BC vs. normal, as in the first segment of ‘B’, otherwise, it is confusing whether the change is really ‘up’ or ‘down’ as in the 2nd and 3rd segments, where it is normal tissue vs. ER+ BC or vs. ER- BC. The 4th segment should be ‘invasive BC vs. DCIS’.

Table 4: It is not clear why there are two different values for the same TFF3 gene. It is probably because of different probes used in the arrays, but it has to be explained.

Level of interest: An article of importance in its field

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:**

I declare that I have no competing interests.