Peer Review File

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Reviewer Comments

Reviewer A

Comment 1: Cholesterol pathway genes have been reported to be upregulated during breast cancer progression (10.1186/s12885-022-09353-2). Incorporate this in the introduction section (line 105/106)

Reply 1: Thank you for the suggestion. I have included the above-cited reference in the revised version.

Comment 2: Dataset, line 126: Please specify the distance of histologically normal tissue from the tumor tissue. Was it adjacent normal (less than 2cm away) or distant normal (more than 2cm away)? More appropriate control would have been normal breast tissue from disease free women as histologically normal tissue is known to already possess molecular abnormalities similar to DCIS.

Reply 2: In principle, I agree with the Reviewer's comment on using normal breast tissue from healthy women as a control instead of histologically normal tissue adjacent to breast cancer, but, as clearly stated in the title, this study aimed to specifically compare the interaction of the cholesterol biosynthesis and Hippo pathway in DCIS and corresponding normal epithelium provided by the same patient; that to avoid potentially misleading biases due to the different genetic profile of individuals and net of common molecular abnormalities.

As regards the distance of the histologically normal tissue from the tumor, the question is not pertinent because, as described in the original article (Breast Cancer Res 11:r17, 2009), laser capture microdissection was used to obtain highly enriched populations of patient-matched normal or malignant epithelial cells, which purity was, then, verified by microscopic examination.

To avoid misunderstanding, I substituted the generic term "tissue" with the more specific "epithelium" in both the title and the revised manuscript.

Comment 3: Need to restructure this section.

Since the study is mostly focused on describing correlation of various cholesterol pathway genes among themselves or with HIPPO signaling pathway genes, it would be beneficial to combine results and discussion section. Start each concept by describing what is the question you are asking, then hypothesizing what is expected, next listing the correlation or no correlation and lastly discussing what that result means (correlation or no association). Do this for all the figures. It will tremendously help readers follow the paper.

Reply 3: I'm very sorry, but the Author Guidelines firmly define that a separate discussion section is required for the original article.

Comment 4: Study lacks experimental validation of their findings. Cell lines could be

used for in vitro validation. You possibly combine datasets DCIS and cancer from one study and normal breast tissue from other studies. These datasets could serve as validation datasets.
Reply 4: Translation of the findings achieved in cell or animal models to humans may be dangerous due to the many misleading associated biases (species, experimental protocols, etc.). Moreover, as explained previously, the present study aimed to compare the interaction of the cholesterol biosynthesis and Hippo pathway in DCIS and the corresponding normal epithelium provided by the same patient. Therefore, it is not correct to combine sets of independent data containing DCIS or normal mammary tissue or use cellular lines stabilized from normal or neoplastic mammary tissues.

Comment 5: You could comment/ investigate if Hippo pathway (because of its inverse correlation with MVA) pathway correlate with resistance to statins (https://doi.org/10.1186/s12885-022-09353-2).
Lastly, need to make a stronger case for what these associations mean and specify future directions.
Reply 5: The suggested investigation of the correlation between the Hippo pathway and resistance to statins is not feasible in the case series analyzed in the present study because no patient was treated with statins.
Conversely, since the study described in the article by Bhardwaj et al. faced the possible emergence of a resistance to statin chemoprevention treatment, a specific mention has been included in the conclusion remarks.

Reviewer B
Comment 1: Line22, what is “such a disruption”?
Reply 1: It refers to the disrupted cooperation between mevalonate and Hippo pathways cited just one line above.

Comment 2: Line 85, there was no evidence of YAP form a complex with TAZ; figure 1, the model of YAP enter nuclear form a complex with TAZ was wrong.
Reply 2: As correctly highlighted by the Reviewer, there was no evidence of a direct interaction between YAP and TAZ. Both factors had an N-terminal TEAD binding domain and a series of serine residues targeted by LATS1/2 phosphorylation (Physiol Rev 94: 1287-1312, 2014). Accordingly, I amended Figure 1 and the text.

Comment 3: Line 139, it is not clear how author measured gene expression, since author claim data was derived from “a dataset publicly accessible at the National Center for Biotechnology Information’s Gene Expression Omnibus database (www.ncbi.nlm.nhi.gov/geo/)”.
Reply 3: Gene expression was evaluated in the original study (Breast Cancer Res 2009;11:R7), and the corresponding estimates, once filtered and $\log_2$ transformed, were stored the at the Gene Expression Omnibus database, making them publicly accessible to the scientific community.

Comment 4: Lack of rational for select BIRC5 and CDK6 as components in Hippo pathway, since they are not typical YAP/TAZ targets.
Reply 4: In contrast to the assertion of the Reviewer, several studies have demonstrated that BIRC5 is a well-established YAP/TAZ target gene (Semin Cell Dev Biol. 23:785-93, 2012; Cell. 151:1457-73, 2012; Nat Cell Biol. 20:888-99, 2018) and CDK6 is a direct downstream target gene of the YAP–TEAD complex (Cancer Res 73: 3615-3624, 2013).
The rationale for including them in the panel of selected genes is based on the crucial role that BIRC5 and CKD6 play in breast cancer initiation and progression. BIRC5 codes for survivin, a well-known antiapoptotic protein overexpressed in DCIS (Br J Cancer 94: 253-258, 2006) and invasive breast cancer (Surg Oncol. 21:125-31, 2012; Int J Mol Sci. 24(14):11827, 2023), used as a prognostic factor (Biosci Rep. 40(2): BSR20193678, 2020; Genet Test Mol Biomarkers. 26(9):411-421, 2022) whereas CDK6 is involved in the physiologic mammary development by controlling negatively the cyclin D-CDK4/CDK6 complex during cellular transition from G1 to S phase (Mol Cancer Res 2: 105-114, 2004).
We added this information in the revised version of the Gene Selection section.

Comment 5: How author conclude from gene expression data to YAP nuclear location?
Reply 5: The conclusion is based on the cumulative evidence that the mevalonate cascade, the core of cholesterol biosynthesis, contributes to the regulation of the Hippo signaling pathway providing the isoprenoids required for GTPase activation, the nuclear accumulation of the YAP/TAZ coactivator, and the subsequent gene transcription and that the disruption of this cooperation associated with tumor progression.