TCR TRANSLATIONAL CANCER RESEARCH
ADVANCES CLINICAL MEDICINE TOWARD THE GOAL OF IMPROVING PATIENTS' QUALITY OF LIFE
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Peer Review File

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Reviewer A

This in silico study of the correlation of CLU with patient survival and tumor cell infiltration and immune markers presents new and interesting insights into the potential contribution of this oncoprotein to the tumor immune microenvironment. However, the manuscript still has several limitations and needs significant improvements and revisions as indicated below.

Comment 1. While the manuscript presents a substantial amount of data, the figure legends lack sufficient detail to assist the reader in the independent interpretation of the results.
Reply 1: Thank you for your comment. We added some explanations to the figure legends to make it easier for the reader to understand.(see Page 18, line 3-4/6-8; Page 19, line 1-3/10-13; Page 20, line 3-5)
Changes in the text: The clearer Figure 4 has been replaced.

Comment 2. Figure 1 is based strictly on CLU transcript expression comparisons between tumor and adjacent normal tissues in different cancer types using the TIMER and TCGA databases. The data does not accurately capture what we know about CLU as an oncoprotein. As an example, it shows that CLU levels are higher in normal vs tumor in PRAD (prostatic adenocarcinoma). While this might be true (although adjacent normal could be molecularly abnormal), CLU has different stress-induced isoforms (secreted vs nuclear) that promote therapy-resistance and other tumor aggressive properties in advanced PRAD and other tumors. Thus, transcript expression data without taking into consideration protein isoform expression data may not accurately predict patient prognosis in different cancer types. This limitation of Fig. 1 is ignored in the Discussion.
Reply 2: Your comments are very important for this study. We have further read and discussed the relevant literature and made modifications in the discussion section.

Comment 3. High CLU expression correlated with increased OS in various tumor types according to Fig. S1 (page 7, lines 11-14). An asterisk or some other kind of

identification mark should be placed on the individual plots for these tumors in order to facilitate data interpretation.

Reply 3: Thank you for your comment. According to your reminder, we have made corrections on the figure.

Comment 4. There appears to be discordance in the breast cancer OS data presented in Figure 2 between the PrognoScan (C) and Kaplan-Meier plotter (N) databases. While the Prognoscan database shows better prognosis in breast tumors with high CLU, the Kaplan-Meier plotter database shows the opposite. How do the authors explain this? This should be clearly discussed given that the conclusion that high CLU expression correlates with better prognosis in BRCA is at the heart of the manuscript.

Reply 4: Thank you for your comment. First, different databases have different data sets, and there may be inconsistent conclusions between them; Second, the data sets used in this study, high CLU expression was associated with a good prognosis of breast cancer in the PrognoScan database (OS HR=0.64, P=0.010; DMFS HR=0.77, P=0.017), and Kaplan-Meier Plotter database showed that the results were consistent (OS HR=0.78, P=0.01; DFS HR=0.73, P= 5E-08).

Comment 5. Page 7, line 17, indicates that "Since we found that CLU expression was associated with good prognosis…" This and many other similar prior and subsequent statements should be modified to indicate whether the authors are referring to "high" or "low" CLU expression.

Reply 5: Thank you for your comment, your comment will give readers a better understanding of the article's intent. We have revised the article accordingly. (see Page 7, line 17)

Comment 6. Following the comment made above in #3, similar markings need to be added to Fig. S2. The authors indicate on page 8, lines 8-11, that CLU expression was significantly associated with different immune parameters in a specific number of tumor types. However, it is very difficult to identify these specific tumor types the way the data is presented. Specific markings will facilitate this.

Reply 6: Thank you for your comment. We have revised the article accordingly.

Comment 7. Also in Fig. S2, the top 5 panels and labels are not as sharp as those in the rest of the figure. This figure would benefit from labeling the different tumor types with larger fonts to the right of each panel. The small fonts used in this figure, combined with the succinct description of the figure in the text, make its analysis very cumbersome.

Reply 7: Thank you for your comment. We have revised the article accordingly.

Comment 8. Given all the information presented in Tables 1 and 2, the values showing statistical significance should be bolded to facilitate analysis.
Reply 8: Thank you for your comment. We have revised the article accordingly (Black bold font in Table 1/2/3).

Comment 9. The legend in Fig. 3 indicates for A-D that "CLU correlates with...". However, a statistical correlation was found only for panel B.
Reply 9: Thank you for your comment. Perhaps our statement was inappropriate, and we have corrected it in the manuscript.

Comment 10. Figure 4 is very hard to interpret since the labels are very small and partially erased.
Reply 10: Thank you for your timely comment. The figure has changed.

Comment 11. The authors argue in the Discussion (end of page 11) that CLU may regulate the immune tumor microenvironment. However, there is no literature discussion to support this argument. In fact, apart from the first 4 sentences (lines 10-15) in the discussion, which cite a few references, the rest of the discussion lacks a meaningful discussion based on the literature regarding possible mechanisms by which CLU may regulate immune infiltration in the tumor microenvironment. No experiments were conducted by the authors to provide mechanistic evidence for an immune regulatory role of CLU in the tumor microenvironment. The authors must consider that the correlations between CLU expression and infiltrating immune cells and markers in breast cancer and other tumor types may not be causative and related to other biological or immune phenomena.
Reply 11: Thank you for your comments. At present, there are very limited studies on the mechanism of CLU in tumors, which is what we need to further study in the future.

Reviewer B

Comment :Yang et al. submitted an original article concerning clusterin and its possible implication in breast cancer prognosis. The authors used several bioinformatic tools to assess the correlation between clusterin and several tumor-associated parameters, including the status of the immune cell. The role of clusterin in tumor development is postulated for several years but still, its clinical potential has not been implemented.

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The authors missed in the Discussion recent papers concerning clusterin and breast cancer:

Bioengineered. 2021; 12(1): 278–285.

Clinical importance of serum secreted clusterin in predicting invasive breast cancer and treatment responses

https://onlinelibrary.wiley.com/doi/full/10.1002/advs.202003205

Inhibition Lysosomal Degradation of Clusterin by Protein Kinase D3 Promotes Triple-Negative Breast Cancer Tumor Growth

https://pubmed.ncbi.nlm.nih.gov/31428526/

Aberrant fucosylation enables breast cancer clusterin to interact with dendritic cell-specific

ICAM-grabbing non-integrin (DC-SIGN)

https://pubmed.ncbi.nlm.nih.gov/28890185/

Stromal Clusterin Expression Predicts Therapeutic Response to Neoadjuvant Chemotherapy in Triple Negative Breast Cancer

It is also important to mention that Clu overexpression is not breast cancer-specific and other cancer types also are associated with sClu:

https://journals.sagepub.com/doi/full/10.1177/10732748211038437

Circulating Clusterin Levels and Cancer Risk: A Systematic Review and Meta-Analysis

The authors suggest in the title that the manuscript describes only breast cancer, but in supplementary results, other cancer types are also analyzed. Please also comment on these results in the discussion in more detail.

Could you hypothesize about the possible background of such differences? Or clusterin upregulation is a universal, tumor-induced phenomenon.

Language should be improved.

Reply: Thank you for your comment. Your comments are very important to this paper, and we have added some literature to the discussion section. (see Page 10, line 10-12/ line 17-20)