Peer Review File

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Reviewer A

Human epidermal growth factor receptor 2 (HER2) protein is overexpressed on the surface of various epithelial ovarian cancer tissues, mediates the proliferation, differentiation, metastasis, and signal transduction of tumor cells, and thus is a potential cancer therapeutic target. In the manuscript "Bioactivity of recombinant humanized monoclonal antibody against HER2 and its mechanism of action in ovarian cancer", authors constructed the light- and heavy-chain expression vectors of rhHER2-mAb, and investigated its affinity and antibody-dependent cytotoxicity (ADCC) efficacy.
Couple questions are required to be answered before it will be accepted.
(1) In the whole text, the "antibody-dependent cytotoxicity (ADCC)" should be changed to "antibody-dependent cellular cytotoxicity (ADCC)".
Reply: Thanks for the suggestion. The errors have been corrected in the revised manuscript.

(2) It was better to add related reference (DOI: 10.21037/tbcr-21-22) about the review of the current landscape and future perspectives of HER2-targeting antibody.
Reply: Thanks for the suggestion. We have added related information and reference in Introduction line 110-122.

(3) The anti-HER2 antibody was the crucial topic in the study. How about the progress of anti-HER2 antibody? Please state in the introduction.
Reply: Many thanks for the reviewer. We have added related information in line 113-117.

(4) In the part of methods, "The plasmid was inserted into the constant regions of the HER2 antibody light-chain (Lc) and heavy-chain (Hc) DNA coding fragments" was wrong. Please revise. And what was the meaning of "a homologous recombination kit"?
Reply: Thanks for your suggestion. We have revised the corresponding errors as "The HER2 antibody light chain (Lc) and heavy chain (Hc) were inserted into the plasmid coding region."in line 144-145 and "one step cloning kit"in line 182.

(5) Why to use two-way ANOVA in statistical analysis? Please state clearly.
Reply: Thanks for your suggestion. Here, an unpaired two-tailed t-test was used to compare differences between two groups. We re-examined the data for significance using unpaired two-tailed t-test. No change in the text.

(6) In the table 1 legend, please supplement the illustration of underline in the tale 1. And supplement the full-names of abbreviations in tables and figures in legends.
Reply: Thanks for your suggestion. We have added related information in tables and figures legends.

(7) The "rhHer2" and "rhHER2" both were showed in the text. Please unify.

Reply: Thanks for suggestion. We have modified “rhHer2”to “rhHER2” in main text, table and figure legend.

(8) Compared to other obtained commercialized anti-HER2 antibodies, what were the advantages of the studied antibody in the research? Please state in the discussion.
Reply: Thanks for your suggestion. Here, we discuss our own sequence obtained from phage library. We used a transient gene expression (TGE) method to produce our antibodies. And, our antibodies have a similar level of affinity with Herceptin and exhibit better activity in vivo compared to Herceptin monoclonal antibodies. We state it in line 448-452.

(9) In the conclusion of discussion, “a mechanism of action through the effective binding of rhHER2-mAb to the HER2 antigen on the cell surface was observed” was showed. But, the mechanism of action was not investigated in the study. It was better to provide.
Reply: Thanks for suggestion, here we using Flow cytometry and SPR confirmed the binding of HER2 antibodies to receptors in figure 4 and table 3.And the vitro activity also shows ADCC effect in Figure 4B. Our antibody acting on the extracellular portion of the HER-2 receptor, it blocks the activation of intracellular tyrosine kinase and inhibits the proliferation and survival of HER-2 dependent tumor cells. No change in the text.

Reviewer B

1) First, in the title please indicate the research methodology of this study, i.e., in-vivo and in-vitro experiments.
Reply: Thanks for your suggestion. We have added related information in title.

2) Second, the abstract needs some revisions. The background did not indicate the question to be answered, the potential clinical implications of this research focus, and what the aim of this study was. The methods need to describe how the TGE method was used and more details of the experiments such as Optimization of light/heavy-chain ratios. The results need to quantify the findings by reporting statistics such as expression levels and P values. The conclusion needs comments for the implications of the findings, not to repeat the significance of this research again.
Reply: Thanks for your suggestion. We have added the aim and potential clinical implication in background in line 30-31. We have added more details of the experiments in methods line 36-39. We have added p value in vivo animal experiments line 47. We have revised the conclusion in line 50-54.

3)Third, in the introduction of the main text, the authors did not clearly describe what the knowledge gap is and what the limitations of prior studies are on the current research focus. Further, it remains unclear what the potential significance of this research focus is.
Reply: Thanks for your suggestion. We have added related information in line 113-129.

4)Fourth, in the methodology of the main text, please have a brief overview of the research procedures of this study and the purposes of these procedures. In statistics, please specify the

details of the two-way ANOVA, i.e., the variables to be compared and what the two grouping variables are. Please ensure P<0.05 is two-sided.

Reply: Thanks for suggestion. In the methodology of the main text, the short title has shown the purposes and the procedures was followed by each part in method. An unpaired two-tailed t-test was used to compare differences between two groups. We re-examined the data for significance using unpaired two-tailed t-test which p<0.05 is considered significant. Line 311.

Reviewer C

1. Figure 1

Please explain Lc and Hc in the legend.

Reply: Thanks for your suggestion. We have added the related information in Figure 1 legend.

2. Figure 2

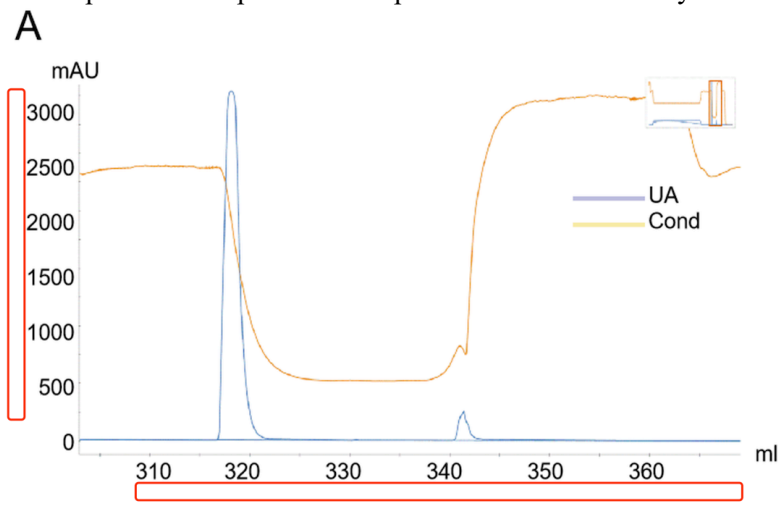
a) Please explain LC and HC in the legend.

b) In figure 1, LC and HC were written as Lc and Hc, please check and unify.

Reply: Thanks for your suggestion. We have added the related information in Figure 2 legend and unify the LC/HC in figure 1.

3. Figure 3

Please provide the specific descriptions of the x-axis and y-axis.



Reply: Thanks for your suggestion.We have corrected the Figure 3A and provided the descriptions of the x-axis and y-axis.

4. Figure 4

Please also provide the legend for A and B.

Figure 4 HER2-high-expression cell lines selection and antibody-dependent cytotoxicity assays of rhHER2-mAb by lactate dehydrogenase releasing assays.

(A) xxx; (B) xxx. rhHER2-mAb, recombinant anti-HER2 humanized immunoglobulin G monoclonal antibody.

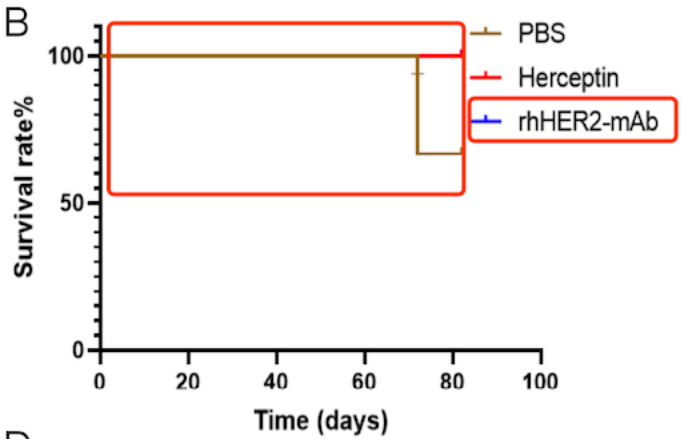
Reply: Thanks for your suggestion. We have added the related information in Figure 5

legend:(A) Expression of HER2 on different cell lines detected by FACS; (B) In vitro cytotoxicity of rhHER2-mAb using LDH release assay.

5. Figure 5

a) Please explain PBC in the legend.

b) rhHER2-mAb was not showed in the curve, please check.



Reply: Thanks for your suggestion. a) We have added the related information in Figure 5 legend. b) The PBS curve cover the rhHER2-mAb curve,we have corrected it in Figure 5.

6. Table 2

Please explain Lc and Hc in the legend.

Reply: Thanks for your suggestion. We have added the related information in Table 2 legend.

7. References/Citations

a) References 4 and 8 are the same, please delete one of them and revise both the citation in main text and reference list's order.

Reply: Thanks for your suggestion. We have corrected References 8 as “Nallet S, Fornelli L, Schmitt S, et al. Glycan variability on a recombinant IgG antibody transiently produced in HEK-293E cells. N Biotechnol. 2012 May 15;29(4):471-6. doi: 10.1016/j.nbt.2012.02.003”.

b) Please double-check if more studies should be cited as you mentioned “studies”.

82 and ovarian cancer cells (1,2). Studies have shown the essential role of HER2 in
83 processes such as tumor cell proliferation, transformation, and invasion, and reported
84 that in addition to being a prognostic indicator, HER2 is also an important therapeutic
85 target for precision treatment (3). HER2 gene can promote angiogenesis and increase

Studies have shown that HER2 siRNA could be a potential drug for the treatment of HER2 positive cancer. Virus-based siRNA and shRNA strategies can effectively solve the problem of siRNA system delivery in vivo, these concerns remain major issues which make it use in clinical regarding immunogenic response and insertional mutagenesis(25). Some studies have shown that co-delivery of HER2 siRNA and

Reply: Thanks for your suggestion. We have corrected the “Studies” to “Study”.