Peer Review File
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Reviewer A

The authors engaged in a non-systematic search develop a review of an important topic. There is no doubt that drug resistance remains one of the most vexing problems in the development of novel and innovative treatments for lung cancer. Despite our greatest attempts to develop innovative therapies, chemotherapy and surgery, mixed with a bit of radio-therapy, remain at the core most treatment regimens. No doubt, targeted therapy and immunotherapy are making headway, but even if this is massively successful, based on the concept of immunotherapy and how it works, it is likely that only a small fraction of the cancer cells will be amenable to such treatments.

Reply 1
We are very grateful for the professional and meticulous review by the reviewers. The original intention of writing this review was to provide a phased summary of the progress of CRISPR-Cas9 technology in lung cancer drug resistance research, and to speculate on its potential development direction based on our own research knowledge and clinical experience. We did not intend to conduct a systematic search to provide a comprehensive overview of the development of a particular research direction, and the description of the search strategy was merely to indicate the main source direction of the content we reviewed. Subsequently, we will follow the guidance of the reviewers to complete the necessary revisions to the manuscript and respond point by point to the reviewers' comments.

Changes in the text: [line 40 to 45]
We have revised the relevant descriptions in the revised manuscript to avoid it as a systematic research review. While ensuring compliance with formatting requirements, we have clarified the main characteristics of the subjects summarized in the review.

I was struck by line 20…. *Non-systematic search of the Web of Science and Pub Med databases*… Exactly what does that mean? Any attempt to provide a review of this important topic needs to be exhaustive and complete, this type of library project doesn't meet the criteria for publication.

Reply 2
In the revised manuscript, we have removed these descriptions and apologize for any misunderstanding caused to the reviewers. As mentioned above, the purpose of this review is not to systematically study theCrispr-Cas9 technique, but merely to summarize the application of CRISPR-Cas9 technology in lung cancer drug resistance research,

especially in targeted drug resistance research, based on our scientific research and clinical practice experience. This issue is an important aspect of clinical lung cancer treatment, especially now, compared to squamous cell carcinoma patients who have widely used immunotherapy. A lot of the adenocarcinoma patients still use targeted therapy as the first-line medication. What`s more, for some patients with rare mutations, targeted drugs can achieve very good clinical effects. However, the problem of drug resistance to targeted drugs are standing in the front of clinical treatment, has become an important reason affecting the overall prognosis of patients. CRISPR-Cas9 gene editing is just one of the many research directions undertaking to overcome drug resistance. We are merely introducing the research progress that we are aware of and that deeply attracts our attention, and trying to elucidate its process and development path based on our research and clinical experience. The entire manuscript does not set any explicit criteria for publication.

Changes in the text: [line 109 to 114 and 121 to 145]
We have reorganized these two paragraphs and added descriptions of the challenges and importance of lung cancer drug resistance research as reviewer`s guidance.

First, the authors should consider describing in greater detail what the challenges are for the treatment of lung cancer. While there is no doubt that the authors weave a bit of this into the text, particularly in the introduction and the early part of the methods, it is not entirely clear. The authors missed most important target gene in this entire field: NLF2L, which encodes the protein NRF2… From a brief literature search of my own, this particular target is the one that is furthest along in developing a novel and inventive approach to reduction of resistance for the treatment of lung cancer. Unless I've missed it terribly, this gene is mentioned only anecdotally and as it relates to KEAP1. Here again we can see the lack of sophistication of this review likely a lack of experienced by the authors in the field; NRF2 is disengaged from its partner KEAP1 either due to the mutations in the NLF2L2 gene or an incredible amount of stress (such as chemotherapy or radiotherapy) which is put onto the tumor cell. This is the foundational pathway or chemo resistance but it is given only cursory treatment. NRF2 is the master regulator that controls resistance to chemotherapy and radiation for lung cancer. The explanation starting on line 363 to roughly line 373 is woefully inadequate. The author should reconsider writing an entire section on this resistance pathway, the mutations in both KEAP1 and NLF2L2 and the work using CRISPR that has not only been published extensively, relating it to the large amount of press that has surrounded this approach. The Zhang lab started this idea many years ago ultimately, they were right, referring to this to this pathway activity …as the dark side of NRF2. Gene repair of mutations in EGFR is a wildly impractical way to use

gene editing or cancer and should be scrubbed from the paper completely. This is where expert I is needed in evaluating means and approaches, as we did not lead a simple list. The only way to overcome drug resistance is to disable the pathway enables tumor cell to acquire it and enables it to continue to function. Again, a big miss by the authors.

Reply 3

The professionalism of the reviewer has deeply impressed me, and we believe that the reviewer must have a very strong research accumulation and professional vision in the NLFL2 field. However, during the revision process, we found that reviewer had a small clerical error of the gene name of NLF2L. We could not find the NLF2L gene in any website or database. According to the reviewer's description that it encodes the NRF2 protein, and this protein is related to KEAP1, we guessed that NLF2L should refer to NFE2L2 (ENSG00000116044), which is the gene that encodes NRF2. Therefore, our response will be based on NFE2L2 in the revised manuscript.

In the manuscript, we have discussed NRF2 quite extensively, and it is presented as a separate paragraph. Based on the reviewer's guidance, we have made a lot of revisions to this paragraph, including clarifying the encoding protein and updating the references. We believe that NRF2 may be a very important potential target in the study of tumor drug resistance. However, since we may not be professional researchers in NRF2, we do not subscribe to the belief that NRF2 is an unparalleled or pivotal gene in drug resistance. It is also possible that we do not have prior accumulation on the function of this gene, so the discussion may not regard NRP2 as a unique important gene. But we have made as comprehensive a discussion as possible according to the reviewer's guidance. We think, due to the different genes that researchers focus on, some other genes have also shown great potential value in lung cancer drug resistance. For example, we cannot agree with the reviewer's mention of EGFR gene repair. In fact, the use of CRISPR-Cas9 to modify lung cancer EGFR gene mutations has been reported in the literature since the advent of CRISPR-Cas9 (doi:10.15252/emmm.201506006). At the same time, compared to the function of a certain gene, we hope to highlight the importance of CRISPR-Cas9 in drug resistance. When designed the review, we mainly referred to another review published in Nature (doi:10.1038/s41568-022-00441-w), which focuses on the application of CRISPR-Cas9 technology in immunotherapy and CAR-T therapy, which are hot topics in tumor research. Compared with these therapies, targeted therapy or its drug resistance research is relatively not so hot, and our review also hopes to introduce more about this aspect, which is also the original intention of our review. It may be in this aspect that the study of CRISPR-Cas9 is still very insufficient, but we hope to introduce all kinds of possibilities, so it also includes EGFR. Therefore, we believe that the statement "wildly impractical way" is inappropriate. At the same time, we also do not agree with "The only way to overcome drug resistance is to disable the pathway that enables tumor cells to

acquire it and enables it to continue to function." According to our clinical experience, overcoming tumor drug resistance requires a multi-faceted research and treatment strategy. The CRISPR-Cas9 we introduced is only a small part of it, and other feasible and effective measures include but are not limited to the development of new drugs, combination therapy, and the use of drug delivery systems, etc. It is not doubt that this is a continuous, personalized process. We have found many signaling pathways to regulate drug resistance, but how can we block these pathways multiple times through this only way? I think this is somewhat biased.

Changes in the text: [line 399, 408, 411]

We have reorganized the descriptions of these sentences and cited new references.

So, in conclusion, essentially what these authors have done is essentially carried out a simple library search without the base knowledge of what is important and what is not. Such literature searches are insufficient and will not advance the field. I recommend complete rejection of the paper, although I would leave the door open if the authors rearrange and focus primarily on the pathway I described above.

Reply 4.

We are very grateful to the reviewer for spending a lot of time and attention to review our manuscript. Although there may be some differences in research understanding between us, the reviewer's professionalism in the field of NRF2 research still impresses us. We also did a lot of searching in this gene and learned a lot of new knowledge. We believe the differences understanding in some views have also made the manuscript more professional. Although we still cannot agree with the inexplicable definition of "library search" and the importance of a single gene in the entire research field (especially since no clinical trials targeting this gene in any solid tumor drug resistance have been found). In our understanding, lung cancer, as a polygenic disease, is far from being cured by regulating the expression of a single gene, and drug resistance involves multiple aspects. Currently, changes based on the patient's personal mutations, key regulation of drug resistance pathways, or even the application of nano-drug delivery systems, are all to solve this problem from multiple aspects. Although we impress the reviewer's high professionalism, we do not agree with the reviewer's arbitrary "in conclusion" for this article, just as we cannot agree with the reviewer's belief that "The only way to overcome drug resistance is to..." and "the most important target gene in this entire field: NLF2L, which encodes the protein NRF2." We hope that the journal can consider our revised manuscript and revision opinions from the perspectives of readability and inspiration.

Reviewer B

The article reads fluidly.

What did the search reveal concerning genes associated with CRISPR, cancer, and glycobiology related pathways that cause MDR?

CRISPR work has been done (some in early in vitro studies) in the realm of glycobiology, specifically with the intention of understanding the role glycan related genes play in MDR. The use of CRISPR was employed. The review would be elevated (and I am encouraging the inclusion of) if it were to include this, perhaps, more niche area of work that has proven pivotal in recent years in our understanding of MDR-to the review. One such recent article is cited below (of course there are more):

Early in vitro evidence indicates that deacetylated sialic acids modulate multi-drug resistance in colon and lung cancers via breast cancer resistance protein
Sec. Cancer Molecular Targets and Therapeutics
Volume 13 - 2023 | https://doi.org/10.3389/fonc.2023.1145333

This would be appropriate to discuss in the heading ###Application of CRISPR-Cas9 in identifying the key genes and molecular
351 mechanisms of lung cancer drug resistance, for example. If the authors are willing to expand the review to include glyco-related work.

Overall, I am an advocate for publication, but I would like to learn more about the focus of the review. Although it isn't feasible to ensure inclusion of ALL work done, the review felt limited compared to my knowledge of lung cancer in the context of CRISPR and mechanistic pathways relating to the regulation of glycans. Please consider expanding the review to include such a section.

Reply:
Thank you for your professional and focused comments. Your expertise in the field of glycobiology has provided us with a deeper understanding of this area. In the revised manuscript, we have cited the relevant reference as per your guidance. However, our knowledge in the field of glycobiology is quite limited, and our work primarily focuses on the editing of well-established and authoritative key genes using CRISPR Cas9 technology to alter lung cancer drug resistance. Therefore, our exploration of the drug resistance mechanism may not be in-depth, and we hope to gain more insights in future research endeavors.

Changes in the text: [line 449 to 465] We added a separate paragraph to describe the glycobiology.