Peer Review File

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Reviewer A

1. The authors did the literature search on December 20, 2022. This was over 7 months ago. It seems to me that this review should be as up to date as possible, and should include publications that have been published after this date, such as: doi: 10.1016/j.celrep.2023.112212. In this more recent publications, a lot of the mentioned challenges are being addressed. For instance, the authors select for p53 wildtype lung cancer organoids that have MAPK pathway mutations by removing EGF from the media and by adding a pan-ERBB inhibitor. The authors also point out that the efficiency of establishing the tumor organoids vastly differs depending on the subtype, and report 78% success rates for small cell lung cancer.

response 1: Thanks for the suggestions. We have updated the review with some important and recent literatures.

Changes in the text: see line 263-282 and Table 3

2. Table 2 seems to be incomplete. Why did the authors select these components, but left out other components mentioned in table 3, such as Prostaglandin E2, Forskolin, or Dexamethasone

response 2: Thanks for the suggestions. We have revised it in the manuscript.

Changes in the text: see Table 2

3. The introduction focuses on NSCLC, but at least one publication (reference 52) also reports data on small cell lung cancer (line 353). The introduction should reflect that not only NSCLC is discussed in the review.

response 3: We have changed the NSCLC to lung cancer in the introduction according to the suggestion.

Changes in the text: see line 69

4. The authors have a section on TIME, but also talk about fibroblasts and other non-immune cells, so the authors are actually talking about the TME (tumor microenvironment). This should be changed.

response 4: Since we introduced some LCOs co-culture systems containing non-immune cells in TME have been introduced in section 5.2, we have changed the TIME to TME in its title according to the suggestion. However, immunotherapy is one of the most important means of lung cancer treatment, so we still paid more attention to the immune cells in this section, which is the key of constructing immunotherapy screening methods derived from LCOs.

Changes in the text: see line 384 and 389-392

5. The term “second-dimensional” should be changed to “two-dimensional” for 2D cultures.

response 5: Thanks for the suggestions. We have revised it in the manuscript.

Changes in the text: see line 74

6. The publication should be checked for grammar, spelling errors, and the usage of correct words (e.g. “appropriate” instead of “approximately”, line 215).
response 6: Thanks for the suggestions. We have revised it in the manuscript.
Changes in the text: see line 261

Reviewer B

The review is quite informative and useful and the science behind is sound. However, it is clear that different sections have been written by different persons, and that is readily noticed in the quality of the English language, which is poor at the beginning of the manuscript but much more correct in the last half. Some changes to English are suggested in the attached file, but much more should be corrected and the authors should revise phrase construction and spelling throughout the manuscript.
response: Thanks for the suggestions. We have revised errors in the use of language in the manuscript.
Changes in the text: see line 74, 208, 214-215, 251, 261 and so on

Reviewer C

The manuscript titled “Promising preclinical models for lung cancer research - lung cancer organoids: a narrative review” aims to narratively review the use of lung cancer organoids (LCOs) as a more effective and holistic model for conducting lung cancer research. The focus of the study revolved around challenges and potential solutions in the generation of LCOs, the relevant applications and accompanying limitations of LCO models, and the future of LCOs in lung cancer research. Overall, there was a lack of coherence and adequate connections should be drawn between the author’s main points to improve the progression and fluency of the review. Please see specific comments below:
All pages – Citations are inconsistent and switch between using a superscript citation and incorporating the author’s name directly into the text. Please address grammar and typographical errors throughout. (i.e., Line 44 “LCOs is” should be changed to the plural “are” instead of “is” and Line 215 “appropriate” should be changed to “approximately”. Typographical error on Line 177.)
response 1: Thanks for the suggestions. We have revised it in the manuscript.
Changes in the text: see line 46, 261and 208

Abstract – Please rephrase the following sentences:
Line 33-34 “However, different histology and genetic background caused the complexity of lung cancer, more efforts should be made to optimize the culture systems and platforms.”
Line 80 “In addition, in vivo patient derived xenograft (PDX) models, generated from patient tumor tissue implantation in immunodeficient or humanized mice, retain tumor heterogeneity and mimic tumor microenvironment better than cell line models.”
response 2: Thanks for the suggestions. We have revised it in the manuscript.
Changes in the text: see line 37-38 and 81-84

3. Methodology of Lung Organoids – The introduction paragraph should only briefly address the aims of the subsections (patient specimen collection, tissue digestion, etc.) without going into too much detail.
response 3: Thanks for the suggestions. We have revised it in the manuscript.
Changes in the text: see line 85-108

3.2 Tissue digestion – Lines 134-138 "Besides, trypsin should be avoided in organoids generation because the expression of cell surface antigens might be altered, leading to free-DNA-induced cell aggregation. On the contrast, the trypLE, an analogue of trypsin, can be used in passaging during LCOs culture without changing the cell surface antigens." Make the connection between avoiding trypsin and using digestive enzymes in the tissue digestion process clearer. Also, what is the consequence of using trypsin on organoid generation and growth? Address the importance of the cell surface antigens.
response 4: Thanks for the suggestions and we have revised it in the manuscript. The choose of digestive enzyme used for detaching cell mainly include trypsin and trypLE (an analogue of trypsin). Although trypsin with stronger enzymolysis effect has limited adverse effects on cell characteristics, the use of trypLE was more commonly, given that its milder effect is benefit to avoiding overtrypsinization.
Changes in the text: see line 147-151

3.4 Verification and characterization of LCOs - Line 185-186 "In addition, in clinical practice, these markers used to distinguish the subtypes of lung cancer, especially for lung adenocarcinoma (LADC) and lung squamous cell carcinoma (LSCC). The former usually have positive TTF-1 and CK7, and p63 and CK5/6 in the latter." Rephrase sentences to provide more clarity between markers distinguishing LUAD from LSCC.
response 5: Thanks for the suggestions. We have revised it in the manuscript.
Changes in the text: see line 212-215

Line 196- 199 "We therefore recommended a complete verification and characterization of LCOs should include the morphological and immunohistochemistry assessment, combined with a genetic profile analysis by CN, WES or RNA sequencing." Are there advantages/disadvantages to each genetic analysis technique?
response 6: Thanks for the suggestions. For the common genetic profile analysis, we have provided additional explanation in the manuscript. Currently, WGS is considered as the most accurate method for identification of the patient-derived organoids due to the access to the whole genome information, but considering the high cost, many researchers have chosen WES as the more cost-effective and less laborintensive alternative. Similarly, detection of commonly mutated genes in lung cancer using a small panel such as EGFR, TP53, KRAS, and so on can save the cost, but some potential discordances between organoid and original tumor may not be found, given that the tumors may not necessarily carry these mutations.
Changes in the text: see line 219-229

4.1 Low Successful Rate… - Line 220-221 "Cancer cells from metastases sites and pleural effusions, can also be used to avoid the overgrowth of normal lung epithelial cells." Address

why these cancer cells from metastases sites and pleural effusions are advantageous compared to other cancer cells in mitigating normal lung epithelial cell growth in LCOs.
response 7: There is no clear evidence at present, so we have revised it in the manuscript.
Changes in the text: see section 4.1

5.2 LCOs for modeling the tumor immune microenvironment – Discuss the primary differences between the two models used to generate a tumor microenvironment in organoid models.
Line 320 – Define "CAF" acronym to avoid ambiguity.
response 8: Thanks for the suggestions. We have revised it and made supplementary explanation about these two co-culture models in the manuscript.
Changes in the text: see line 424-426

Reviewer D

The English language of the manuscript is in need of major review. Overall, the concept is ok, though the authors missed a rich literature on murine tumoroids, and human and murine normal lung organoids that could help to frame the ideas better.

Here is a brief review of the English changes to make before I realized there were too many for me to review:
Line 27 "mimic the microenvironment of" or "mimic microenvironments of"
response 1: Thanks for the suggestions. We have revised it in the manuscript.
Changes in the text: see line 32

Line 29, organoids have not developed at a fast pace, but the field of research has,
response 2: Thanks for the suggestions. We have revised it in the manuscript.
Changes in the text: see line 34

Line 29, "and these models"
response 3: Thanks for the suggestions. We have revised it in the manuscript.
Changes in the text: see line 34

Line 30, "several studies have reported protocols for"
response 4: Thanks for the suggestions. We have revised it in the manuscript.
Changes in the text: see line 35

Then I jumped ahead….

Line 209-210, "is that normal lung epithelial cells in the culture often grow faster than the lung tumoroids"
response 5: Thanks for the suggestions. We have revised it in the manuscript.
Changes in the text: see line 251-253

Reviewer E

The review by Chen et al. tries to provide an overview of the lung cancer organoid field. Although numerous reviews have been written about this subject in the past years, the review of Chen et al. distinguishes itself from the others by focusing more on the technicalities of patient-derived organoid generation and cultivation. Hence, this review deserves a spot in literature. However, several major issues need to be resolved before it can be considered for publication:

1) It was impossible to correct all grammar errors so the entire manuscript should be revised, preferably by a professional team. Grammatical errors can be found in amongst others: sentence structure, use of singular/multiple, verb conjugations, incorrect use of terms, use of italic and inconsistent use of full versus abbreviation.

response 1: Thanks for the suggestions. We have revised it in the manuscript**.**

2) Although the use of 2D cell line-based cultures is briefly discussed (lines 70-79), the authors immediately shift to 3D patient-derived cultures as a better alternative while they entirely skip the field of 3D lung tumor spheroids based on cell lines. This should be included (e.g.: https://pubmed.ncbi.nlm.nih.gov/34950592/ - Biomedical Applications of Non-Small Cell Lung Cancer Spheroids AND https://onlinelibrary.wiley.com/doi/full/10.1002/anbr.202100124 - Novel 3D Lung Tumor Spheroids for Oncoimmunological Assays).

response 2: We have supplemented the relevant introduction of 3D cell culture in the introduction section as a transition to the development of organoid technology.

Changes in the text: see line 85-92

3) This review is based on papers found via the search terms ‘lung cancer’ and ‘organoids’. As many other lung organoid-related reviews have been published in 2023 (https://www.frontiersin.org/articles/10.3389/fbioe.2023.1205157/fullhttps://www.frontiersin.org/articles/10.3389/fbioe.2023.1205157/full - Patient-derived organoids of lung cancer based on organoids-on-a-chip: enhancing clinical and translational applications AND https://www.frontiersin.org/articles/10.3389/fphar.2022.1083017/full - Open questions in human lung organoid research AND https://www.frontiersin.org/articles/10.3389/fbioe.2023.1132940/full - Footprints: Stamping hallmarks of lung cancer with patient-derived models, from molecular mechanisms to clinical translation), authors should

a) update the review with all papers (research papers and reviews concerning lung cancer patient derived cultures for (pre)clinical applications found until Aug 2023)

b) include more search terms as they appear to have missed numerous papers that discussed the use of lung cancer patient-derived cultures (e.g. https://pubmed.ncbi.nlm.nih.gov/33218948/ - 3D In Vitro Model (R)evolution: Unveiling Tumor-Stroma Interactions AND https://pubmed.ncbi.nlm.nih.gov/28202521/ - Ex Vivo Explant Cultures of Non-Small Cell Lung Carcinoma Enable Evaluation of Primary Tumor Responses to Anticancer Therapy AND https://pubmed.ncbi.nlm.nih.gov/29101162/ - Ex Vivo Profiling of PD-1 Blockade Using Organotypic Tumor Spheroids AND https://pubmed.ncbi.nlm.nih.gov/32034078/ - Use of Ex Vivo Patient-Derived Tumor Organotypic Spheroids to Identify Combination Therapies for HER2 Mutant Non-Small Cell Lung Cancer AND

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8464600/ - Human tissue cultures of lung cancer predict patient susceptibility to immune-checkpoint inhibition AND https://pubmed.ncbi.nlm.nih.gov/26088102/ - Towards personalized medicine: chemosensitivity assays of patient lung cancer cell spheroids in a perfused microfluidic platform)

response 3: Thanks for the suggestions. We have updated the review with some important and recent literatures.

Changes in the text: see Table 1

4) As comparisons of culture systems/LCO generation methods are the most 'novel aspect' of this review, they should be discussed/compared more in depth (not only success rate and applications) in terms of size of organoids, culture period in days/weeks, mono-or multi-cellular culture, plastic recipient, ECM component, type of analysis to define 'success' of organoid generation,… I also suggest to put more emphasis on this 'LCO generation part' of the review by changing the title.

response 4: Thanks for the suggestions. There is lack of relevant researches on the influences of a single factor on the specific growth characteristics of organoids. But we have modified the section 4 in the manuscript, which discuss the specific problem during LCOs generation including the choose of conditioned medium, maintenance of the tumor heterogeneity, and long time for LCOs generation.

Changes in the text: see section 4

5) Message on line 215 'appropriate 50-60% of lung cancer patients harbor TP53 mutation' is needlessly repeated on line 231/232.

response 5: Thanks for the suggestions. We have revised it in the manuscript.

Changes in the text: see line 261

Reviewer F

It is an informative review. The authors focus on lung cancer organic, but more challenging and basic issue would be lung organoid, which is also useful for the study of carcinogenesis (especially incipience of carcinogenesis). Maybe the readers would enjoy on the information of organoid of lung itself in one paragraph before the authors' discussion going to the focused cancer models.

If one of the authors have a piece of their original picture of lung cancer organoid, the reader would be more impressive.

response: Thanks for the suggestions. Although our team is struggling for LCOs culture, we do not have suitable original picture data included in this review.

Reviewer G

This manuscript by Chen & Ye et al. provides a concise summary of the current state of understanding of lung cancer organoids as a promising preclinical model for lung cancer research. The authors have explored the methodology for organoid generation as well as the

characterisation and application of this model. I would argue that the innovations in this manuscript are extremely limited and have concerns over the novelty of this Review compared to existing publications in the field.

Main comments:
The authors provide a broad, but simple, overview of LCO culture and their application. The following points could be added in order to strengthen the existing manuscript.

Comment 1: The search criteria should be expanded to include original articles published up until September 2023. There are many studies relevant to this review which have been published after December 2022.
response 1: Thanks for the suggestions. We have updated the review with some important and recent literatures.
Changes in the text: see Table 1 and Table 3

Comment 2: The authors have not mentioned alternative 3D tumour modelling methods for example, spheroids or explants. Whilst understandably the main focus of the manuscript is LCOs, it is important to consider the advantages and disadvantages of such systems in comparison with organoids, especially when discussing their clinical applications for drug screening.
response 2: Thanks for the suggestions. We have compared the advantages and disadvantages of the common preclinical models including 2D cell culture, spheroids, organoid and xenograft in section 5.1 in order to show the applicability of LCOs in high-throughput drug screening.
Changes in the text: see line 310-357

Comment 3: The inclusion of table 2 and 3 give an extensive overview of the culturing conditions used for the establishment and maintenance of LCOs. It would be useful to integrate these more into the text of section 3.3 as currently they are somewhat stand-alone. Perhaps proving a few examples from each table in the text would help reinforce the authors' message of the inconsistency and complexity of LCO culture media.
response 3: Thanks for the suggestions. We have modified section 3.3 and reinforce the inconsistency and complexity of LCOs culture media in section 4.1.
Changes in the text: see line 157-168 and 250-283

Comment 4: The lack of optimisation of LCO culturing conditions and media are heavily repeated throughout the manuscript. In line with comment 3 above, authors should integrate the culturing media information from sections 3.3 and 4.2 to avoid repetition and for an easier understanding by the reader.
response 4: Thanks for the suggestions. We have modified it in the manuscript.
Changes in the text: see line 157-168 and 250-283

Comment 5: The authors mention the use of WES and RNAseq in section 3.4. Whilst this enables the robust characterisation of LCOs, these methods are low-throughput and expensive.

Authors could mention additional cost-effective methods for the molecular characterisation of tumours e.g. qPCR.

response 5: Thanks for the suggestions. We have modified it in the manuscript. WGS is considered as the most accurate method for identification of the patient-derived organoids due to the access to the whole genome information, but considering the high cost, many researchers have chosen WES as the more cost-effective and less laborintensive alternative. Similarly, detection of commonly mutated genes in lung cancer using a small panel such as EGFR, TP53, KRAS, and so on can save the cost, but some potential discordances between organoid and original tumor may not be found, given that the tumors may not necessarily carry these mutations.

Changes in the text: see line 219-229

Comment 6: The authors have mentioned the use of tissue from metastatic sites and pleural effusions as a source of cancer cells to derive tumour organoids. Whilst there is a high success rate for the establishment of these organoids, it is important for the authors to also discuss the disadvantages of these models. One example, among others, is that these organoids do not largely reflect the primary tumours of these patients and are more useful in metastatic studies.

response 6: Thanks for the suggestions. There must be differences among using different tissue types for establishing patient-derived organoid models, but it seems to be not that concerned. In the researches previously reported, the patient-derived organoid models were often established by different tissue type in a single research, where the identified accordance between LCOs and original tumor is considered acceptable for recapitulating primary tumor. So, we don't plan to discuss the disadvantages of these models in this review.

Comment 7: Authors should discuss the high tumour heterogeneity of lung cancer and how LCOs cannot fully reflect the disease in vivo, even if organoids are established from different parts of an individual tumour. This is extremely important when assessing the efficacy of LCOs as a drug screening tool and such limitations have not been discussed thus far.

response 7: Thanks for the suggestions. We have added section 4.2 to discuss on the maintenance of the tumor heterogeneity.

Changes in the text: see line 284-296

Comment 8: The authors provide a good overview of the tumour immune microenvironment in section 5.2 but only discuss the relevance of LCOs as a tool for testing immunotherapies in their conclusion. The observation that the PD-1/PDL-1 axis in cancer organoids strongly reflects that of the primary tumour in vivo has been a hot topic in recent years. The use of LCOs as a prediction platform for responsiveness to immune-checkpoint inhibitors should be discussed in the main text of this manuscript.

response 8: Thanks for the suggestions and we have provided additional explanations. Although these LCOs platforms have been confirmed with capacities of predicting the responsiveness to immune-checkpoint inhibitors and considered with huge potential for clinical translation, they still need more clinical data to support it.

Changes in the text: see line 415-423

Comment 9: To use of genetically engineered organoids, edited using novel CRISPR-Cas9 technology, to study the effects of specific KRAS and additional oncogenic mutations could be included under section 5 of this manuscript.

response 9: Thanks for the suggestions. Although genetical editing based on CRISPR-Cas9 has been used in the organoid researches of other tissue type, but those used in LCOs is too few. So, we don't plan to add a separate section for it