Peer Review File

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Reviewer A

1. Grammatical change - In the first paragraph (Background), last sentence change part to "portion". In methods add "our" before own work.
   Reply: Changed

2. The key content and findings section needs a lot of work, and the conclusion needs work both in punctuation and sentence structure. To start, the first sentence needs to be broken up, it is not understandable as it stands now. The presence of CTCs means the disease is metastatic. Do you really mean therapies should not be given to patients with metastatic disease? Yes, there are many cases when a tumor is removed the patient is cured. Metastatic disease requires more intense treatments, but therapies can extend quality and quantity of life.
   Reply: No one means that SCLC should not receive chemotherapy.

3. Techniques to capture CTCs is no more tedious than to separate CD8 or CD4 or NK cells etc. All these sections need work.
   Reply: For sure, the rare CTC cells with a frequency as low as a few cells among 10 million leucocytes are much more difficult to separate and to characterize due to the lack of specific markers separate from the disputed EpCAM.

4. CTCs can be used for much more than to indicate metastatic disease.
   Reply: The use of CTCs to monitor the course of the disease and response to treatment is mentioned.

5. Geonomics on the solid tumor will uncover the existing mutations but does not tell you which mutations are being used.
   Reply: It is pretty clear in SCLC that this tumor is driven by the inactivation of p53 and RB1 tumor suppressor genes in combination with epigenetic changes.

6. Line 100-101 -What is the prognostic threshold – explain
   Reply: Explained, number of CTCs in 10 ml blood discriminating a good from an adverse prognosis.

7. Overall what is the purpose of the paper? There is a lot of data taken from the many, many references but what is the take home message?
   Reply: Due to the scarcity of CTC cell lines many questions regarding invasion, survival in the circulation, extravasation, role of EMT, prognostic significance, … and others have not been resolved. This review summarizes the existing data and point to the most likely explanations for these metastatic processes.

Reviewer B

This is a narrative review of the significance of circulating tumor cells in lung cancer. It goes into detail from the basics to its clinical significance and I think it's a very useful review, but there are some criticisms.

Major

1. Title: "Significance of Circulating Tumor Cell in Lung Cancer: A narrative review" is preferable.
   Reply: Changed.

2. Methods is not in the text. It is desirable to specify the specify process, indication criterion, and exclusion criterion and make the flow chart leading to the selected paper into a figure.
   Reply: Pretty clear: PubMed and Euro PMC were searched from January 1st, 2015 to September 23th, 2022 using the following key words: “NSCLC”, “SCLC”, “CTC” and “Angiogenesis” and supplemented by data from our own work.

3. Private data that has not been accepted in a paper is presented, which is undesirable. If it is shown, it should be done in Methods. Also, the title should be “Significance of Circulating Tumor Cell in Lung Cancer: a modified narrative review”
   Reply: MANUSCRIPT IS IN PRESS.
   Suggestion 1 for title seems appropriate.

4. “Angiogenesis and CTCs” session should be moved before SCLC and CTCs because they are not just a concept for lung cancer.
   Reply: Changed

Minor

1. Figure 1; Circulating Tumor Microembolus (CTM) should be Circulating Tumor Microembolus (CTM)/Clustered Circulating Tumor Cells (CTC).
   Reply: Corrected

2. Line 158; I think it would be better to give a brief description of disseminated tumor cells (DTCs).
   Reply: Included

3. Line 303: PDX is the first occurrence and spell out is required.
   Reply: Changed

4. Conclusion should be changed to Summary.
   Reply: Changed

Reviewer C

In this review article, Hamilton and colleagues provide a well-documented analysis of the scientific literature and their own data on the significance of circulating tumor cell (CTC) use in lung cancer and some other malignancies. They focus on their implication in the metastatic

process, CTC functional studies and the role of angiogenesis in CTC dissemination.

Major comments:
1. It is paramount that the authors specify the objective of this review article as it is too broad and not very clear throughout the article. They may focus their review more on the role of neoangiogenesis in CTC dissemination.
Reply: This MS discusses neoangiogenesis in the context of the general topic of CTCs.

2. For example, the part on CTC enrichment and detection methods is not necessary and can be removed, as this topic has already been extensively reviewed elsewhere (for example https://doi.org/10.1038/nrc3820).
Reply: This article has been published in 2014 and it is essential to represent the newest developments.

3. The abstract as it stands is not clear regarding the aim of this review; the "background and objective" part does not clearly state the objective (lines 15-26), which makes the rest vague. The "key content and findings" part is not very clear. The authors mention their work in the establishment of SCLC CTC lines but do not clearly position it among the existing scientific literature. Transitions should be revised.
An introduction to the notion of angiogenesis (line 42) /angiogenic switch (line 84) should be added in the abstract.
Reply: The terms neoangiogenetic and MVD describe the situation.

4. The authors should cite more updated literature in the field of CTC research:
- A number of groups have investigated EMT in CTCs most notably the C. Dive team (PMID: 21356352) and the Farace Lab (PMID: 21970878) among others.
Reply: The Dive team have published in 2011 (Updated literature!): Hou JM, Krebs M, Ward T, et al. Circulating tumor cells as a window on metastasis biology in lung cancer. Am J Pathol. 2011 Mar;178(3):989-96. doi: 10.1016/j.ajpath.2010.12.003. This publication has already been cited here as ref. 33 and it states that "Potentially, CTM could reflect the intravasation of tumor cells that had migrated collectively and entered the blood stream via the "leaky" and chaotic tumor vessels that are a feature of highly angiogenic tumors" and that "metastasizing cells adopt some mesenchymal features (eg, expression of vimentin and neural cadherin) but retain some epithelial characteristics (eg, cytokeratin and membrane E-cadherin)". This does´nt mean a real full EMT spindle-shaped mesenchymal cells proposed to cross the strorma.
PMID: 21970878. The group of Farace demonstrated "hybrid CTCs with an epithelial/mesenchymal phenotype in patients with NSCLC". Partial expression of characteristics of cells have experienced EMT, no proof of this phenotype involved in intravasation.

5. When mentioning CTC-derived xenografts (CDX) on page 7, the authors cite only the biobank established in SCLC but 5 CDX models now exist in NSCLC (Morrow et al Annal Oncol 2016, Tayoun & Faugeroux et al 2022)
Reply: The work by Morrow reported "One clinical case study of a NSCLC patient with

advanced metastatic disease. Whereas size-based CTC enrichment revealed abundant heterogeneous CTCs of which ~80% were mesenchymal marker vimentin positive. CDX-positive. These CTCs were separated by a filtration technique known to result in the recovery of a lot of non-relevant cells. This single example is hardly representative of the characteristics of CTCs.
The work of Tayoun T, Faugeroux V, Oulhen M, et al. describes several cases of NSCLC CDX and CDX and "unravel DDR and genome integrity-related defects as a central mechanism underpinning metastatic potency of CTCs" that is not really related to the key steps in the biology investigated for lung cancer.

6. The latest and highly relevant randomized controlled clinical trial "STIC CTC" is not cited PMID: 33151266 and deserves to be mentioned page 11 line 448. It demonstrated the role of CTC count in guiding therapeutic choice in metastatic breast cancer
Reply: Breast cancer is not the topic of this review and, therefore, this important Publication has not been considered.
Massagué J, Obenauf AC. Metastatic colonization by circulating tumour cells. Nature. 2016 Jan 21;529(7586):298-306. doi: 10.1038/nature17038. PMID: 26791720; PMCID: PMC5029466.
CTC clusters have a superior ability to seed metastasis in experimental models[61].

7. The authors mention cfDNA (page 3 line 123) without first introducing it.
Reply: Corrected.

8. Pioneer work on metastasis such as that of J. Massague team may be cited when going over the metastatic process.
Reply: Ganesh K, Massagué J. Targeting metastatic cancer. Nat Med. 2021 Jan;27(1):34-44. This team describes CTC clusters in experimental models and still propose EMT/MET involvement in metastasis than seems not to apply in huma tumor models.

9. Finally, the authors should review the general tone of their writing – for example, page 5 lines 204-205 "lack indicator of the lack of detailed knowledge" – especially in the conclusion part (lines 588-590; 608-609).
Reply: Original sentence: "The frequently used term "shedding" for this release of tumor cells into the circulation is an indicator of the lack of detailed knowledge". No duplication of "lack".

Minor comments
A global revision of spelling and vocabulary used should be performed
- Parsortix™ is spelled incorrectly on page 3 line 121
- A word is missing on page 4 line 141 "their high metastatic…"
- Review the whole sentence lines 187-188 on page 5
- The definition of a CTC cluster is repeated at page 12 lines 497 and 500
Reply: Corrected accordingly.