

Comments. Manuscript 55375:

Reviewer#05106340

Comments #05106340

SPECIFIC COMMENTS TO AUTHORS

In this manuscript, the authors aimed to explore the scenario of CTCs' behavior in tumor (T) and blood in eighteen patients with brain tumors by assessing protein expression. The article is interesting. However, there are still some problems need to be resolved, as detailed below. 1. It is not easy to understand article results and abstract results. the writing of this article should be improved. the key data in this article should be showed in abstract results.. 2. CTC is very rare in the blood, accounting for only about one billionth of the total PBMCs. How did the author judge the protein expression on CTC without cell purification?

Answer to comments #05106340

Comment 1.

It is not easy to understand article results and abstract results. the writing of this article should be improved. the key data in this article should be showed in abstract results.

Response to comment 1.

The revised Result section for abstract is inserted in the Results section of abstract in page 4.

Comment 2.

CTC is very rare in the blood, accounting for only about one billionth of the total PBMCs. How did the author judge the protein expression on CTC without cell purification?

Response to comment 2.

According to the published papers and the reports of related companies the CTCs are rare in the blood. But, there is some challenges in this regard including: 1) the analyzed CTCs are not recognized by the diverse intensity of the target proteins; and 2) this problem is also observed for Flow cytometry in which the negative gates also include the cells with low and medium expression, but they are considered as “negative”; these population of cells play a critical role in evolution. In the present manuscript the degree of intensity is categorized as 1, 2, and 3. Furthermore, in our manuscript adequate tumor cells are enumerated either in the tumor mass, or in the blood stream (as CTCs)

[illegible]

Reviewer: #04089095

Comments: #04089095

Dear reviewer: Thanks for devoting your time to review our manuscript and your valuable comments are highly appreciated.

SPECIFIC COMMENTS TO AUTHORS

This manuscript was aimed to explore the scenario of CTCs' behavior in tumor (T) and blood to provide the hypothetical-based agenda in the brain neoplasms. Protein expression (PE) is assayed by Immunofluorescence in tumor (T) cells and CTCs in eighteen patients with brain tumors. Ratio test was applied between the tumor cells and CTCs, and also in vascular system. Somatic/genomic Ratio- based classification of PE in CCL2(C), VEGF (V), EGF (E); CD13/Cyclin E/Neuronal marker (NM)/, KRT19/ CD45 were investigated. This paper is innovative and written smoothly with clear thinking and rigorous logic, but there are some minor problems: 1. In the second paragraph of the "RESULT", should the word " by" be removed? 2. On page 3, line 7, the name of the person is misplaced. 3. On page 3, line 14, should the word "plan" be replaced by

“planning”? 4.The number of specimens selected is relatively small, which can not accurately represent the individual, and can not accurately reflect the general characteristics of the population. You should collect as much information as possible. 5.The contents of ‘MATERIALS AND METHODS’ are a little too concise. In "Analytical strategy", we should explain in more detail how data analysis is carried out.In “The experimental methods”,the experimental method needs further explanation. 6.In the sixth paragraph of the “RESULT”,you should give a brief introduction to how the data in the table are obtained. Overall, I think this article has certain innovation, but there are also some problems that need improvement. I think this article needs to be reviewed again after revision.

Dear reviewer: Thanks for devoting your time to review our manuscript and your valuable comments are highly appreciated.

****The revised items are highlighted as Yellow.**

Comment 1: In the second paragraph of the “RESULT”,should the word “ by” be removed?

Response to comment 1: the word “ by” is removed.

Comment 2: On page 3, line 7, the name of the person is misplaced.

Response to comment 2: Sorry, I could not find it.

Comment 3: On page 3, line 14, should the word “plan”be replaced by “planning”?

Response to comment 3: In core tips, the word plan is replaced by planning.

Comment 4: The number of specimens selected is relatively small, which can not accurately represent the individual, and cannot accurately reflect the general characteristics of the population. You should collect as much information as possible.

Response to comment 4:

As the number of analyzed cells played the key role in this single cell- based research, the focal population is considered as the total cell numbers, through which the course of evolution could be explored by unmasking the heterogeneity and diverse pattern of protein expression in the single cells.

The text in materials and methods (page 7/1st paragraph) is revised.

Comment 5: The contents of 'MATERIALS AND METHODS' are a little too concise. In "Analytical strategy", we should explain in more detail how data analysis is carried out. In "The experimental methods", the experimental method needs further explanation.

Response to comment 5:

The required texts are inserted within the page 7. The full Information is provided in the legends of Figures 1 and 2 (page 23)

Comment 6: In the sixth paragraph of the "RESULT", you should give a brief introduction to how the data in the table are obtained.

Response to comment 6:

A highlighted text is inserted in the 1st paragraph of result (page9).

For Table 1: an additional column as “Other complications” has been inserted in which more Information

Regarding the clinical complications have been provided.

[illegible]

Comment 2. You need to provide the grant application form(s) or certificate of funding agency for every grant, or we will delete the part of "Supported by Tehran University of Medical Sciences, Research deputy under project No.:32208-30-04-95."

Response: I have stated regarding the project In page 9: "*Ethics and consent declaration:* The present study was approved and granted by the deputy of Research, Tehran University of Medical Sciences (TUMS) (32208-30-04-95). The written consent declaration

There were no other grants. Besides, I have provided the ethics certificate in which this project (32208-30-04-95) is also mentioned.

With sincere appreciation

Parvin Mehdipour