Reviewer's report

Title: Selection of Cancer Stem Cells: a Role in Acquisition of Resistance to EGFR Inhibitors in Lung Cancer

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Reviewer: Raj Batra

Reviewer's report:

Ghosh et al present an important report to add to the sparse literature on “Cancer Stem Cell” (CSC) properties in lung cancer. They utilize a paired model of H1650 and H1650 EGFr-blocker-resistant cells to characterize “CSC” properties. The rationale for the study is that clinical treatment of EGFr-blockers is transiently effective in selective subsets of patients with EGFr mutations, and that the recognized mechanisms for that resistance need to be better understood. They potentially have a model in which they can deduce those mechanisms.

The MAJOR issues and inquiries I have with the report are as follows:

MATERIALS AND METHODS:

1) I believe that the cell model used (H1650 cells) are derived from an advanced stage lung cancer model (a malignant pleural effusion). If that is the case, please discuss that derivation phenotype might impact the outcome measures where one is counting aggregates in suspension or anchorage independent growth as functional attributes of “CSC”?

2) With respect to spheroid formation assay, how were cells dissociated for serial transfer?

3) With respect to soft agar assays, what was the “growth medium” used for infiltrating the agar?

RESULTS AND DISCUSSION:

4) The text on page 8 does not corroborate the reported observations in Figure 1A.

5) Exactly what is being measured in figures 1A and 1B? How are these measures normalized? If these are measures related to qRT-PCR, then please include the primers used and data analyses undertaken to make and report these measures.

6) With respect to Figure 1c, please provide the rationale for using beta-catenin (as opposed to E cadherin) membrane localization as a measure of epithelialoid properties. Please discuss whether anything be deduced from the absence of nuclear b-catenin in 1C?

7) With respect to Figure 1D, please describe the details of how the motility assay was conducted (it is not described in methods).

8) With respect to Figures 2, 3, 4 and 6, can one derive a standard error about
the mean (SEM) from 2 measures?

9) Again, exactly what is being measured in Figure 2B and how was this normalized? (I suspect that constitutive expression profiles are being compared, but neither the primers used for amplification nor the analytical details for normalization are presented).

10) What does the author mean by the term "self-renew"? Is this term synonymous with the ability to efficiently form soft agar colonies? and/or with possessing the ability to serially form soft agar colonies? And if so, then what is the molecular basis for verifying this as a “self-renewal” property?

11) What is the basis of making the statement that culture in RPMI 1640 with 10% FBS induces "differentiation”? Are adherent cells more "differentiated" than an "aggregate cluster" of cells in suspension?

12) What is the surface phenotype of the parental versus erlotinib resistant cells with respect to CD44/CD24 status?

EDITORIAL COMMENT (DISCRETIONARY)

1) It would be interesting (and perhaps predictable) that the changes induced by drug exposure may contribute to therapeutic resistance above and beyond erlotinib exposure. The paper would be made much stronger by the inclusion of such data.

The discussion needs to be significantly bolstered.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:

I declare I have no competing interests.