Author’s response to reviews

Title: Rapid chemokinetic movement and the invasive potential of lung cancer cells; a functional molecular study

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Author’s response to reviews: see over
Dear Editor,

We are pleased to hear from you regarding our manuscript “MS: 6378777248751053”, and below is our response to outstanding concerns from the reviewers and the editorial board. A change to the manuscript is highlighted and detailed in Bold.

Yours sincerely,

Lilian Soon

REVIEWER 1
The editorial board has adequately addressed most comments from this reviewer. See also response under Editorial Board.

REVIEWER 2

- In the revised form, authors addressed most of comments appropriately. However, a table of representative differentially expressed genes between CON and KINE should be added for readers who investigate the molecular process of chemotaxis and chemokinesis in motility and invasion of cancer cell.

The complete microarray analysis that includes differentially expressed genes will be sent to readers upon request.

REVIEWER 3

- Still remains an excessive title.

Title Page and Title:
The title has been slightly modified from “Rapid chemokinetic movement correlates with the invasive potential of lung cancer cells; a functional molecular approach” to “Rapid chemokinetic movement and the invasive potential of lung cancer cells; a functional molecular study”.

EDITORIAL BOARD

- The authors only performed one array experiment. While certainly not ideal, as long as different RNA preparations were used for the chip hybridization and the RT-PCR (this should be confirmed by the authors
and if they used the same RNA then the validity of the array is questionable), the subsequent confirmation of changers suggest that the data are meaningful and support the conclusions.

The expression of ROM and other genes were verified in RT-PCR experiments using a set of RNA preparations different from that used in the array.

- I would like to know whether the phenotypic differences were stable or drifted. The latter is more likely and could have been assessed directly following non-selective culture and a repeat of the migration assay. However, I would recommend acceptance with clarification of the outstanding queries.

We have previously observed that the unselected CON cells have a small population that are KINE-like in appearance (amoeboid). This may be due to drifts in phenotypes in the CON cell line where the majority of cells are mesenchymal and the minority, amoeboid in appearance. In the selected KINE population, we observed that the weighting of phenotypes is reversed where the majority are amoeboid in appearance with a small mesenchymal-like subpopulation. Under non-selective culture conditions we have not observed large drifts in the phenotype of KINE cells. These cells were shown in Boyden chambers and by time-lapsed microscopy to demonstrate chemokinesis (Figure 1b and Table 2). Therefore, it appears that in this case, there exist permanent genetic changes that stably altered the cell phenotype. Having said that, cancer cells generally have unstable genomes such that under different circumstances, for example, very long term culture and/or in vivo, larger phenotypic drifts may occur.