Reviewer's report

Title: A Novel Circular Invasion Assay Mimics in vivo Invasive Behavior of Cancer Cell Lines and Distinguishes Collective and Individual Cell Motility in vitro

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Reviewer: Jean-Marie Zahm

Reviewer's report:

The manuscript by Kam et al describes a novel in vitro assay, which is claimed to better mimic the in vivo invasion mechanism. The authors show that when compared to the classical wound healing assay, the in vitro circular invasion assay should allow discriminate more precisely the invasive behavior of cells. The manuscript has merits, however the following concerns should be addressed:

Major compulsory revisions

1. Background needs revision according to current published studies on 3D in vitro models of cell migration and invasiveness (apart from the Boyden chamber techniques, several papers describe 3D culture models mimicking invasiveness).

2. Since the overlaid of the wounded cultures with matrigel is a key feature of the circular invasion assay, the authors should add details concerning this technique: type of matrigel (standard or growth factor reduced), volume of matrigel overlaid onto the wounded culture, final depth, time of polymerization...

3. The morphological alterations in cell morphology induced by LPA do not appear as evident as underlined by the authors (figure 4A). Could the authors explain why they used a fourth cell line for these experiments instead of testing the effect of LPA on the 3 cell lines that were used to compare CIA and CWA methods? To improve the hypothesis that CIA could be more informative when comparing collective or single cell migration, it should be of interest to compare the effect of LPA by using CWA method.

4. One point remains unclear in the CIA method: do the cells migrate within the matrigel or do they migrate at the interface between the matrigel and the culture dish substratum?

5. Could the authors give some explanation about the increased reproducibility that they observed with the CIA method?

6. An important point is lacking in the discussion: why is the CIA technique more appropriate to discriminate between different cell lines? Are they some factors at the molecular level which could be involved?

Minor essential revisions:

1. There is a discrepancy between the figure numbers presented in the result section (from 1 to 4) and the current figure numbers (from 1 to 8).

2. Scale bars have to be presented in the microscope images with the
corresponding length in the figure legends.
3. There is no need to present the table with the numerical data in the figures (the mean values are given in the result section.

**What next?:** Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

'I declare that I have no competing interests'