Reviewer's report

Title: A new assay for measuring chromosome instability (CIN) and identification of drugs that elevate CIN in cancer cells

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Reviewer: Rene Medema

Reviewer's report:

In the manuscript entitled "A new assay for measuring chromosome instability (CIN) and identification of drugs that elevate CIN in cancer cells" Lee and colleagues describe a new technique to assess the amount of chromosome missegregation based on the loss of a Human Artificial Chromosome (HAC) and quantification by flow cytometry. In addition, the authors apply this new methodology to determine the rate of missegregation after treatment with "anti-mitotic" drugs. The development of new assays to impartially address the rate of CIN is needed to standardize CIN measurements and for high throughput screenings, therefore the method brings an important contribution to the field.

The manuscript is clearly written; the hypothesis and methods are well presented. The abstract includes an overview of CIN and aneuploidy that helps to introduce the reason for the development of the HAC loss assay, and also includes the results of the measured CIN rates after drug treatment.

Major Compulsory Revisions:

1 - The proposed assay is based on the quantification of the EGFP intensity coming from the artificial chromosome. Although the high frequency for HAC missegregation (10%) confers sensitivity to the assay, it also over-estimates the rate of normal chromosome missegregation. This reviewer would like to see this issue addressed in the text, and if possible a correction factor should be introduced in the mathematical model in order to have a more realistic approximation of CIN rate for the endogenous chromosomes.

2 - In the methods is stated that FACS profiles were acquired 14 days after treatment to quantify HAC loss. The justification was that EGFP protein half-life is one day, and distinguishable differences between treated and untreated samples were only found 10 days after drug washout. It is not completely clear why this is happening. Is it an inherent limitation of the technique due to low EGFP loss, or is the assay measuring the cumulative effects of chromosome missegregation after drug treatment? The authors should provide the FACS profiles of cells before, after drug treatment and different time points after washout.

3 - HAC missegregation has no toxic effects, however loss of one or more chromosomes may increase CIN and compromise cell viability. The mathematical model used to calculate CIN rates assumes that the drug effect is limited to one cell cycle, and that spontaneous HAC loss after drug exposure
does not change. If that was the case, distinguishable differences should be observed two days after treatment (considering EGFP half-life). The loss of HAC is proposed to measure CIN rates, but taking into account that cells are analysed 14 days after drug washout, it suggests that the assay is measuring viable aneuploid cells. In fact, micronuclei formation assays 20 hours after drug treatment don’t corroborate the calculated CIN rates. Especially for nocodazole and peloruside A treatments the differences are quite significant, indicating that HAC loss is measuring viable aneuploid cells. The authors should carefully address this point, by showing total chromosome number before and after drug treatment within different time points. This quantification is essential to demonstrate that chromosome loss is limited to the first cell cycle, thereby validating the mathematical model.

Minor Essential Revisions

4 - Nocodazole is misspelled on tables 1, 2, 3, and on figure 4.
5 - Docetaxel is misspelled on figure 4.
6 - Apoptosis is misspelled in the results section.
7 - On figure 5, in the equations related to F2 generation what is mentioned as P1 should be P2.

Level of interest: An article of importance in its field

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.