Reviewer’s report

Title: FKBPL and its peptide derivatives inhibit cancer stem cells and breast cancer metastasis by downregulating DLL4 and Notch4

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Reviewer: Jean-Philippe Brosseau

Reviewer’s report:

This is a follow up manuscript on the biology and mechanism of FKBPL and peptide derivatives in breast cancer by the Robson lab.

Concern 1: The peptide AD-01 and its effect on endocrine therapy resistance (Figure 5-6) were covered in McKeen CancerRes2010, migratory properties (Figure 3) were covered in Yakkundi PLoSOne2013 and mammosphere formation/cancer stem cell properties were covered in McClements ClinCancerRes2013 previously by the same group in breast cancer cells. In fact, some very similar figure panels were published elsewhere by the same group [Fig. 1A, B; this study vs Fig. 4B; (McClements ClinCancerRes2013)]; [Fig. 3A; this study vs Fig. 3B (Yakkundi PLoSOne2013)] removing some luster on the originality of this manuscript. The true novelty here gets down to the attenuation of breast cancer metastatic progression through DLL4 and NOTCH4 by FKBPL.

Concern 2: The mechanism of action postulate by the author (through modulation of DLL4 and NOTCH4) is highly speculative and solely rely on correlative experiment. Thorough rescue experiment should be carried out to conclude that FKBPL inhibit CSC and metastasis by downregulating DLL4 and Notch4. From the data presented, DLL4 is at best a potentially good marker that correlate with AD-01 treatment / FKBPL level.

Concern 3: The experiment in Figure 3 C-D is interesting but required further attention to conclude. In fact, the reason why two different set of experiments (panel C with pre-treatment and panel D without pre-treatment) is presented. Does the FKBPL actually modify the metastatic microenvironment before cancer cells reach it? Does it interfere with cell grafting? Also, the in vivo experiment relies on bioluminescence as a single readout which is a surrogate for the number of cancer cells. The author should complement this figure by measuring tumor growth with other readout such as tumor weight, tumor volume estimate.

Concern 4: Overall, the figure flow, their legends and data presentation are difficult to follow

- The knockdown validation is presented in the middle of the figure (Fig.1, 2, 4).

- Labels on x axis should be something like: control, FKBPL overexpression. Not the name of the cell line and a code like A3, D2, etc... Also please indicate what is the negative control used in all experiments (PBS?, empty vector?)
- Figure 3C and D should be separated in individual panel and presented/discussed in the main manuscript

Concern 5: There is a couple of citations that are duplicated in the reference section. Please double check all references as each should have a unique number.

Other comments

-In Figure 2C and 4D. Please indicate which band is the correct one in the NOTCH4 ICD blot

-In Figure 2D. Could the authors comments on the CD44 KD not having an impact by itself (comparison between both control in panel D). Statistic should be provided between the two treated condition (the authors provide stats between control and treated but not between treated and treated)

-According to the data presented, FKBPL/AD-01 influence the % of stem cell-like cells population. Did the authors test the impact of AD-01 on "pure" stem cells and differentiated cells as sorted by FACS?

-What are the best evidences indicating that AD-01 is not a prodrug? (that a smaller peptide is not the active substance?)

-In introduction, Page 4, line 35. Please precise which cohort are you discussing and add the appropriate reference

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

No

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

No

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

No

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.
I am able to assess the statistics

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