Reviewers report

Title: Lipid Raft Disruption by Cholesterol Depletion Attenuates Migration, Invasion and Angiogenesis by Downregulation of uPAR and MMP-9 in Breast Carcinoma Cells

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Reviewer: Charlotte Morrison

Reviewers report:

Re: ‘Lipid Raft Disruption by Cholesterol Depletion Attenuates Migration, Invasion and Angiogenesis by Downregulation of uPAR and MMP-9 in Breast Carcinoma Cells’

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BMC Cancer

The authors have used methyl beta cyclodextrin to disrupt lipid rafts in 2 breast cancer cells to investigate the effect that disruption has on MMP-9 and uPAR expression and resulting effects on breast cancer cell migration and angiogenesis.

The authors present co-localization data to show that uPAR and MMP-9 co-localize with lipid raft markers - the data for ZR 751 isn’t as convincing and in both cell types the vast majority of both proteins isn’t co-localized with lipid rafts and this isn’t addressed. The authors don’t address the fact that MMP-9 is a secreted protein a small fraction of which appears to be associated with lipid rafts, and switch between looking at MMP-9 levels in cell lysates and supernatants.

Treatment with methyl beta cyclodextrin is shown to effect cell migration, invasion and angiogenesis using in vitro assays

Major Compulsory

1. Toxicty assay method is given and ‘non-toxic’ levels mentioned but data is not shown for methyl beta cyclodextrin toxicity, since many results could be explained by general toxicity I think this data should be shown.

2. Data is referred to without showing the data:

Results

Para 2. Lysates from different time point ranging from 1-24 h (only 1 and 24 h are shown)

Para 4 Time kinetics are referred to for MMP-activity in MDA when only 1 and 24 h are shown
Para 8. Total Akt and P13-k not shown as stated in text and fig legend.

3. There seem to be insufficient controls for western blots and zymograms
   For example:
   Fig 1 &2 westerns D & E
   2 westerns were shown of lysates from 2 time points 1 and 24h, GAPDH was
   used as a loading control but only 1 GAPDH blot was shown, was this the
   loading control from 1h or 24 h? Both need to be shown.

4. The westerns are all cropped, there are no positive controls and there are no
   molecular weight markers - how do you know or how can readers judge if the
   antibodies are specific?

5. Zymos again no positive controls for uPA or MMP-9
   Throughout the paper the MMP-9 zymo is used to infer that it is a measurement
   of MMP-9 inhibition. This is actually only a measurement of the level of MMP
   present not even its activity, since pro-MMP-9 will also generate a band of
   clearing on a zymo and there is no positive control to show where pro and active
   MMP-9 would run, no inference about MMP-9 activity can be made.

6. Fig. 2 Zymograms, are not quantitative since they do not have a linear
   read-out and there are no loading controls

7. Presumably the best western blots are shown and the quality of some of the
   blots shown is not sufficient to perform the quantification that is presented,
   For example:
   Fig 2.D 1h untreated-there is a big non-specific blob
   Fig8 B lane 3-half the band is missing

8. Para 4 percentages given in text don’t match the figure

Minor Essential Revisions

1. Abbreviations are often used without defining e.g. MMP, uPAR, LR etc

2. “As previously shown” is used at the start of nearly every section in results
   without a reference or Figure number-it would aid clarity to add these

3. The clarity of the paper could be enhanced both :
   - 1 h and 24 h time points used are presumably post the 1 h treatment time ?
     That isn’t stated

4. Results par 9
   Typo “with” missing

5. Figure and Figure legends clarity:
   Fig 2 has the wrong antibody
Fig. 4 A & B are in the wrong place at the beginning and in the middle
Which time point is shown for the RT-PCR?
This is not real-time RT-PCR, data it is semi-quantitive RT-PCR
Figure 4 are these fractions or a pooled fraction-not clear from text, legend or figure
Fig 6. Data should be presented in the order discussed to avoid confusion, legend confusing as jumps around.

Level of interest: An article of limited interest

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