Author's response to reviews

Title: Effect of staurosporine on the mobility and invasiveness of lung adenocarcinoma A549 cells: an in vitro study

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Author's response to reviews: see over
We would like to thank the reviewers for their insightful comments and have done our best to answer their questions.

Point by point response to reviewer’s comments

Reviewer's report:

This manuscript by Wang et al., was written in a neat manner and results are novel and presented in a good way. But including the following corrections will increase the quality of the manuscript.

1. In the first line of the abstract change the sentence to “Lung cancer is one of the”
   
   Correction made.

2. In the Methods section of the abstract check MMP-9 and give the abbreviations for LnR, MMP-9 and uPA.
   
   Correction made.

3. Again in the introduction section change first line change it to “Lung cancer is one of the”
   
   Correction made.

4. Since both MMP-9 and uPA are cytoplasmic proteins why the authors didn’t counterstained the slides with any of the nuclear stain for example DAPI. By doing this way they can increase the quality of the presentation.
   
   We agree that a DAPI counterstain may improve the effectiveness of the image. However, the addition of an additional fluorescent dye could interfere with the quantification of the target fluorescent signal, which was why this counterstain was not done.

Reviewer's report:

In their manuscript, Wang et al describe phenotypic changes induced in A549 lung adenocarcinoma cells treated with staurosporine. Using in vitro assays, the authors address changes in cell adhesion, migration and invasion, as well as expression levels of molecules known to be involved in these processes. Others have previously reported effects of staurosporine on this particular cell line and staurosporine analogues have been used in clinical studies of lung cancer patients. However, the authors suggest that their results are promising in the design of novel strategies that would affect the development of lung carcinoma. Since similar studies have been published previously, perhaps the authors could elaborate more on how their studies differ from these earlier reports, and how their results could be used to develop novel strategies that would affect lung carcinoma development.

   We have addressed this in the Discussion section.

Major compulsory revisions:
1. In the abstract, the authors state that all experiments were conducted for 24 h, however the cell adhesion and migration assays were conducted for shorter durations, while the cell invasion assay was longer. 
Corrected.

2. Figure 1 shows images of A549 cells that have been treated for 24 hours with either DMSO or increasing doses of staurosporine. With the higher concentrations, the cells appear to be fewer in number and more rounded. By electron microscopic analysis shown in Figure 2, these rounded cells show changes attributed to apoptosis. Since the results from the adhesion, migration and invasion assays could just be a result of cells undergoing apoptosis, the authors should include assays that quantify the amount of apoptosis induced in the staurosporine treated cells. Also, how does staurosporine affect the proliferation of A549 cells? 
We have added the results of the MTT assay and FACS apoptosis assay where we show the quantification of cell proliferation in the absence and presence of staurosporine and the quantification of apoptosis in the absence and presence of staurosporine.

3. The authors state that intracytoplasmic levels of MMP-9 and uPA in A549 cells decreased with staurosporine treatment, but this is difficult to assess from the images shown in Figure 5. Additional images of higher magnification should be shown. Also, is this effect at the protein or RNA level? More convincing data should include Western analysis. 
We do not have a higher magnification image available. The results shown are from an immunofluorescence experiment and hence represent protein levels. Since we have convincing quantitative results of the fluorescent signal (Table 3) the additional western data may not be necessary.

4. Since staurosporine has such a broad effect, perhaps the authors could also use a more specific PKC-# inhibitor in comparison, and in order to better justify their suggestion that staurosporine inhibition of A549 cell adhesion, motility and invasiveness was possibly due to PKC-# inhibition. 
We have included this as a limitation of the study in the Discussion section.

5. At the end of the third paragraph of the discussion, the authors state that staurosporine can regulate receptors on the cell membrane through the inositol phospholipid pathway, yet they provide no evidence of a change in inositol phospholipid signaling. The authors should amend this statement or provide evidence that this is the case. 
We have removed this sentence.

6. Similarly, at the end of the fourth paragraph of the discussion, the authors make another generalization stating that because their results show staurosporine inhibits integrin #1 expression in A549 cells and that the adhesion of these cells to the extracellular matrix is prevented, thus the distant spread of
tumor cells is inhibited. While the authors do show that levels of integrin #1 are affected, they provide no evidence that the adhesion of A549 cells to the extracellular matrix is prevented, or that the spread of tumors is blocked.

*We agree and have removed this sentence.*

7. The preceding sentence of the fourth paragraph of the discussion should be clarified. What “transcription factor” are the authors referring to?

*As Rigot et al showed, PKC may activate the adhesion of cells through phosphorylation of integrin, increased integrin levels on the cell surface and reconstruction of the cytoskeleton. Moreover, PKCs were able to activate the insulin-like growth factor I signalling pathway and led to MAP kinase activation and to the induction of cell migration. [16].*

8. Some of the English could be improved, particularly in the materials and methods section.

*Modifications made.*