Reviewer's report

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

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**Reviewer:** Caroline Heckman

**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.

**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.

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?

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.

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.

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.

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.

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

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

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

**Quality of written English:** Needs some language corrections before being published

**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.