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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Version: 5 Date: 14 April 2009

Author’s response to reviews: see over
We would like to thank the reviewers for their helpful and constructive critique of our manuscript entitled “Effect of Staurosporine on the mobility and invasiveness of lung adenocarcinome A549 cells: an in-vitro study”.

We have addressed all their concerns and are convinced that our results along with the edited version of the manuscript will prove to be of interest to the readers of BMC Cancer.

**Point by point response to the reviewers comments:**

In their revised manuscript, Wang et al have added additional results including those from cell survival/proliferation assays and cell cycle plus apoptosis quantification by FACS analysis. While these results could improve the overall study, they are added to the end without any comment on how they impact findings from the other assays. These are functional results that would probably be better suited towards the beginning of the results section along with results describing changes in cell adhesion, mobility and invasion.

We have moved the results “Effect of staurosporine on cell proliferation and apoptosis” such that they follow the results from the cell adhesion, mobility and invasion experiments. We have also incorporated a brief discussion of the significance of these results into the Discussion section.

Since the authors state that apoptosis of STS treated cells peaked at 24 h, this could mean that less invasion is observed because there are fewer cells surviving at the point when invasion was measured.

It is true that at the highest dose of staurosporine used (100mmol), the results of our invasion experiments could have been influenced by the apoptosis caused by the drug. However, at the lower staurosporine dose (1mmol), where apoptosis caused by the drug was not significant (See current Figure 2, the MTT assay), cell mobility and invasion were diminished by 15% and 16% respectively. This was statistically significant (See current Table 2). Thus, our current data clearly demonstrate that staurosporine causes an inhibition of cell mobility and invasion independent of the induction of apoptosis. However, we recognize that a limitation of the present study is the difficulty of excluding the effect of apoptosis while quantifying the inhibition of invasion and mobility by the drug.

In addition, without a nuclear counterstain in the images shown in Figure 5, it is impossible to determine if decreased fluorescence is due to fewer cells, particularly for the staining of MMP-9. As I stated previously, results from Western analysis would be more convincing.

We have presented the quantification of the fluorescent signal as the mean fluorescent intensity of 500 cells in five different visual fields of 200X magnification (100 cells were counted in each field). We believe that this approach largely excludes the effects of decreased cell numbers due to apoptosis. We do agree that cells undergoing apoptosis could contribute to a decrease in the fluorescent signal. However, a Western blot would generate the same questions on decreased cell viability contributing to the lower signal intensity. We agree with the reviewer that future studies on the effect of staurosporine on inhibition
of invasion should address the issue of apoptosis induced by the drug and the resulting decrease in cell numbers.