Reviewer’s report

**Title:** Low concentration of cycloheximide promotes C6 glioma cell cycle arrest at G1 and S phases and cAMP-dependent cell differentiation

**Version:** 2  **Date:** 23 June 2010

**Reviewer:** Ayhan Bilir

**Reviewer’s report:**

1-Inappropriate title: Title should be “Low concentration of cycloheximide induces cell cycle arrest and differentiation at C6 glioma” According to this, running title should be “low concentration of cycloheximide at C6 glioma”

2-Language should be edited. Examples from abstract are defined below: Sentences should be grammatically well constructed.

“Although extensively used as a reagent to inhibit protein synthesis in mammalian cells, how cycloheximide impacts on cell cycle and cell differentiation in cancer cells, to our best knowledge, has not been reported.” “Using the well-established C6 glioma cell line, we found that treatment of C6 cell with low concentration of cycloheximide inhibited cell proliferation and depleted cells at both G2 and M phases, suggesting blockade at G1 and S phases.”

“Marker” should be “marker”

They should add p values to abstract for statistical analysis.

3-Cell line: C6 glioma was the rat derived established cell line. It should be good to define this drug’s effects at human derived cell lines as T98G glioma or others.

4- Background should be written again.

They defined differentiation as defense program of cells as apoptosis to stop tumorigenesis. Differentiation of tumors against chemotherapy lead to resistance. This ability especially seen at stem cells which were 0.4 % at glioma cells. By writing this I exclude dedifferentiation or trans differentiation which is different from differentiation. As differentiation mostly accepted as the part of drug resistance process authors should define in detail how recent publications were used this process against cancer, which mechanisms were involved, is it successful for all cancer types etc.

- They wrote that CHX has dual effect at cancer as proliferation versus inhibition or inhibition versus proliferation (this is biological effect) so if its defined, what they added new to literature. They should emphasize this issue. Their hypothesis should be added otherwise it seemed “fishing”.

- For differentiation evaluation GFAP levels and correlation with cAMP levels were not enough.

5- Materials and methods section

. Cell culture and reagents Rat C6 glioma cell (ATCC cat. number CCL-107) was
cultured in DMEM (Invitrogen) containing 10% FBS. CHX, 8-para-chloro-phenylthio-cAMP (8-pCPT-cAMP) and Adenosine (Ado) were obtained from Sigma. Companies and/or trademarks should be written in paranthesis.

-Evaluation of cell morphology should be defined in detail as steps of fixation, staining…

- The methods should be written in order: cell proliferation results should be written first, colony forming should be written at last. First you define your hypothesis by using cell number then you go colony forming process.

- Immunohistochemical process authors should write dilution rate of antibodies. They should write companies in that form Santa Cruz, State, Country. In addition they should indicate how many cells were seeded on coverslips for IHC process.

- Western blotting analysis used antibodies, their dilution and their trademarks should be written. We also need to know type of the gel (continue or discontinue) and gel concentrations for each parameter.

- Authors should write experiments’ intervals for each procedure. Did they analyze C6 glioma cells’ responses for 24 hour and/or 72 hour?

- #statistical analysis should be added as a section. They only mention the number of experiments at cAMP analysis. We need to know sample number (n:?) and set of experiments for each procedure.

6- Results

- Dose response details should be written in Materials and Methods section. They should indicate IC50 values at results section. What was the cut off value of CHX?

- They should write morphological examination apart from cell proliferation section. This should be new section. To make cell proliferation results they should use terminology as decrease/increase.

- They detect dead cells with Hoechst stain. They didn’t give any numerical data. Also it’s important to define type of the cell death and flow ctometric Annexin-V-FITC or acrdine orange can be useful for this data.

- Colony forming results should be written apart from cell proliferation and morphological section. Colony forming results should be defined in numbers/or graph.

- At western blotting analysis they wrote “from C6 cells untreated or treated with CHX (0.5µg/ml) for 24 or 48 h and carried out western blotting analysis” time should be definite. At figures it’s clear that they did it for two hours but in text body it’s not clear. Western blotting semi-quantitative analysis so they can explain results numerically formulated as protein intensity/actin intensityx100. Western blotting should be also written as individual section.

- Cell cycle results should be given in numeric data, they should not define it only with histograms

- “LCC does not induce apoptosis in C6 cell but depletes cells at G2 and M
phases” was wrong title. Define type of the cell death and flow cytometric Annexin-V-FITC/PI or acridine orange can be useful for this data. They should not say the type of cell death was apoptosis only by evaluating cell cycle or only parameters p53, pAkt. Other parameters as caspase-3, cytochrome-c-oxidase should be also evaluated.

- “CHX-promoted C6 cell differentiation is associated with an increase in intracellular cAMP and can be blocked by adenosine.” This section should be part of Discussion. Only results should be explained.

- I didn’t see p values, I don’t know these data were statistically significant or not. This is a big problem.

7-Discussion
- They should discuss all their data and connect them.

- CHX-promoted C6 cell differentiation is associated with an increase in intracellular cAMP and can be blocked by adenosine.” This section should be part of Discussion.

- They should add compare their results with more manuscripts. References for discussion were not enough. They only use them for introduction.

- What does this sentence mean?

“Since CHX is one of the most used reagents in molecular and cellular biology research and C6 cell is a well-established cell line to study cancer cell proliferation, apoptosis, and differentiation, our findings reported here carry added significance.”

They should emphasize their authenticity and importance of their work for clinic. Last sentence makes his investigation ordinary.

8-References
References should not be written bold.

9-Figure Legends
They are very long, this sentences should be take part in materials and methods section. Because no statistic they are not enough for me to evaluate correctly.