Reviewer’s report

**Title:** Knocking down CDK4 mediated the elevation of Let-7C suppressing cell growth in nasopharyngeal carcinoma

**Version:** 1  **Date:** 20 June 2013

**Reviewer:** Konstantinos Mavridis

**Reviewer’s report:**

The present article by Liu Z. et al describes some aspects of the CDK4-let-7c interactions in nasopharyngeal carcinoma and analyzes CDK4 expression in this malignancy. Extensive revision is needed before this article is suitable for publication. Major and minor concerns are listed below:

**Major Compulsory Revisions**

1. The authors should analyze the expression of let-7c in all NPC tissues and evaluate its prognostic properties; this will strengthen the present study.

2. Details are missing regarding sample collection, storage and preparation for analysis. What is more, since the tissue specimens are FFPE tissues, how was it possible to proceed to miRNA expression analysis without including a proteinase digestion step to remove methylol cross-links between RNA and proteins?

3. P value for survival analysis is missing. Additionally, how was the cut-off value chosen for dichotomizing CDK4 expression values into high/low expression?

4. The authors should proceed to univariate and multivariate Cox regression survival analyses.

5. More details regarding the calculations used to analyze the data of qPCR experiments should be provided.

6. The authors mention that “Knocking down E2F1 by specific siRNA-E2F1 elevated the expression of Let-7C.” How can this be explained? In an article by Bueno MJ et al (Mol Cell Biol. 2010 Jun;30(12):2983-95) it is shown that E2F1 induces the expression of let-7 microRNAs.

7. It is mentioned that let-7c decreases the expression of CDK4 and decreased CDK4 expression leads to downregulation of E2F1 which in turn leads to let-7c overexpression. Can this positive feedback loop be biologically relevant? The authors should discuss this finding, mentioning similar examples from the literature.

8. English language should be comprehensively checked throughout the manuscript; some parts are really hard to follow.

**Minor Essential Revisions**

9. The term “Let-7C” should change to “let-7c” or “miR-let-7c”.

10. Abstract, Methods: Does the term “nasopharyngeal tissues (NPs)”
correspond to normal/unaffected nasopharyngeal tissues? This should be also clarified throughout the manuscript.

Discretionary Revisions

11. Introduction: It is mentioned that: “Synergetic effects of viral infections, genetic alterations, and environmental factors are thought to be the key factors driving the aberrant activity of a variety of genes and signal pathways, such as EZH2, HDGF, EMT-associated genes, cell cycle, and epidermal growth factor receptor signaling pathway et al, which caused the pathogenesis of NPC” This information is too general. The authors should try to be more precise and provide the most salient examples.

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