Author's response to reviews

Title: Knocking down CDK4 mediates the elevation of let-7c suppressing cell growth in nasopharyngeal carcinoma

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Author's response to reviews: see over
Dear reviewer and editor,

We sincerely appreciate your taking time to provide your comments and feedback. Following your suggestions, we have revised the paper. We hope that our revision is what you want.

**Question 1:** The authors have included T classification, N classification, M classification and at the same time TNM stage at their multivariate logistic regression models. By definition, these variables are bound to present significant correlations between them. It is known that this approach introduces collinearity and multicollinearity in the regression models and can seriously affect the interpretation of a model. I suggest that the authors include the TNM stage variable instead of separately including T classification, N classification and M classification.

**Response:** Thanks reviewer for his good suggestion. Indeed, the introduction of separated T, N, M classification will affect the multivariate result. According to the reviewer’s suggestion, we reanalyzed the multivariate logistic regression models in deleting the variables of T classification, N classification and M classification. This result showed that high expression of CDK4 indicated a tendency as an independent prognostic factor ($p=0.087$).

**Question 2.** Taking into consideration that radiotherapy-treated patients present a statistically significant difference in overall survival, the authors should proceed with subgroup survival analysis in radiotherapy treated and non-treated subgroups of patients, with the perquisite that the sample size for these subgroups is adequate.

**Response:** According to the reviewer’s suggestion, we proceeded with subgroup analysis in radiotherapy treated and non-treated subgroups of patients, the result showed that the cumulative survival of NPC patients with the treatment of radiotherapy was higher than those of non-treated group ($p=0.001$)(Figure 5C). This result had added into result section.

**Question 3.** The treatment plans that were followed should be described. The numbers of radiotherapy and chemotherapy treated patients should be included in the clinicopathologic characteristics table (Table 2).

**Response:** According to the reviewer’s suggestion, we supplemented the delineation of radiotherapy and chemotherapy for NPC patients including the numbers of radiotherapy and chemotherapy-treated patients in Sample collection and cell culture of Materials and Methods. However, it seems the numbers of radiotherapy and chemotherapy-treated patients were not fitted for adding Table 2. The Table 2 described the correlation between the clinicopathologic characteristics and expression of CDK4 protein from NPC patients who did not accept any treatment of radiotherapy and chemotherapy.
**Question 4.** It should be made clear throughout the manuscript that let-7c expression analysis was made in fresh NPC and NP samples and not in the FFPE tissues.

**Response:** According to the reviewer’s suggestion, we clearly write that let-7c expression analysis was made in fresh NPC and NP samples but not in the FFPE tissues in the result section (“Let-7c is downregulated in NPC”) of the article.

**Question 5.** In order to use the “2 –##Ct” method for the calculation of gene expression the same sample, usually referred to as the “calibrator” should be included in each run, in order to be used for normalizing the expression units of samples assessed in different runs/plates. It is not clear whether this approach has been followed here. Did the “the sample with the maximal #Ct value” play the role of the calibrator by being included in each run? Also, “2 –##Ct” should be corrected to “2 –##Ct”.

**Response:**

1. In this study, the procedure of real-time PCR for examining the expression of let-7c is as follows: 1) Firstly, we examined the expression of let-7c and U6 in 56 NPC samples and 15 nasopharynx tissues in 2 96-plate. 2) Further, the same experiments were continued to repeat two runs. Thus, our real-time experiments for let-7c and U6 examination were three runs. 3) In each run, let-7c expression was normalized to U6 in each sample. Thus, there were 71 “#Ct value” (56 NPCs+15 NPs) produced in each run. The sample with the maximal #Ct value in 71 samples plays the role of the calibrator in each run.

2. “2 –##Ct” should be corrected to “2 –##Ct”: According to the reference (Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method.) Methods. 2001;25(4):402-8), 2 –##Ct was corrected to 2(-Delta Delta C(T)) in the article.