Author's response to reviews

Title: Therapeutic effects of lentivirus-mediated shRNA targeting of cyclin D1 in human gastric cancer

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Author's response to reviews: see over
Dear Editor

Thank you so much for your detail revises on our manuscript. I changed it according to the reviewers’ comments, and the details are as follows.

Reviewer 1

Reviewer's report:
Results demonstrated by Jin-Hee Seo et al are straight forward and support justification of further testing with lentivirus mediated shRNA cyclin D1. However, a great deal of preclinical work is needed for conclusions stating that this may have potential in clinical development. Such limitations need to be mentioned in discussion (i.e. long term effect of lentivirus exposure, risk of lentivirus DNA integration, off target integration, along with validation of shRNA knockdown activity starting with target RNA and protein as well as downstream signal response). Moreover, future steps involving further animal efficacy related to longterm survival and toxicology/biodistribution should be mentioned as necessary steps prior to IND need to be brought up as necessary prior to possible clinical discussions.

Answer: Thank you so much for your excellent comment. I added lines 8-11, Page 18 and reference.

Other minor comments are included:
Page 10
Line # 4 “…the tumors were injected…”: would be helpful to better describe injection routine (i.e. central single injection, radial injection…)
Was the entire tumor injected?
Answer: I revised lines 4, Page 10.

Did follow up biopsy acquire tissue from the injection points?
Answer: We did not biopsy.
Is there any information on the molecular results of CCND1 knockdown (i.e. protein, RNA knockdown of CCND1, effect of related signal patterns to CCND1)?

**Answer:** I added lines 19-21, Page 16 and reference.

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**Reviewer 2**

**Reviewer's report:**

Many studies have reported that cyclin D1 are closely related to some kinds of cancers, and effected tumor growth, metastasis and drug resistance. The authors try to develop a new therapeutic strategy for treating gastric cancer. So the therapeutic effect of lentivirus-mediated shRNA targeting of cyclin D1 (ShCCND1) was analyzed both in vitro and in vivo. The data showed that lentivirus-mediated ShCCND1 effectively inhibited growth of NCI-N87 cells derived cancer both in vitro and in vivo. The efficiency of shRNA knockdown and variation in the degree of inhibition is mediated by different shRNA sequences and cancer cell lines. The results suggest the possibility of developing new gastric cancer therapies using. The data from the study revealed some evidence to use lentivirus- ShCCND1 for treating gastric cancer. But the manuscript has some shortage needed to be explained and discuss.

1. In the study, only one gastric cancer cell line was used to test in vitro and in vivo, the conclusion needs to be confirmed by the other gastric cancer cell lines.

**Answer:** Thank you so much for your excellent comment. I added lines 19-21, Page 16 and reference.

2. To validate the efficiency of shRNA knockdown on cyclin D1 levels, the results showed ShCCND1-1 and ShCCND1-2 did not decrease cyclin D1 levels. However, ShCCND1-3 resulted in lightly decreased cyclin D1 level compared to NCI-N87, ShCCND1-1 and ShCCND1-2. The cyclin D1 protein did not decrease significantly in Figure , too. The biological effects were also significant in suppressing tumor growth, apoptosis in vitro and in vivo. So I suggest the authors to try more effective ShCCND1.

**Answer:** I added the decreased protein level, 31%, Page 12.

3. In the results, “the cell apoptotic rate was 61.7% in ShScramble and 88.4% in ShCCND1”. The apoptotic rate was 61.7% in ShScramble group, meaning that the apoptosis resulted from
the other factor, not from ShCCND1. And the above apoptotic rates did not matched the values in Figure 3B.

**Answer:** I changed apoptotic rates, 29.19% and 48.14%, page 13, as shown Figure 3B. And I added line 26, Page 8.

I would like to thank to the reviewers and editor. If there are any questions, please contact to +82-2-2049-6113 or yangkyuc@konkuk.ac.kr

Sincerely yours

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