Author's response to reviews

Title: Combined ANT2 shRNA and hNIS radionuclide gene therapy generates enhanced antitumor effects through phenotype modulation of cancer cells and increases CTL immune response simultaneously

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Author's response to reviews: see over
Dear Editor

We are pleased to resubmit our manuscript titled “The combination of ANT2 shRNA and hNIS radioiodine gene therapy increases CTL cytotoxic activity through the phenotypic modulation of cancer cells”. We have performed additional experiments (clonogenic assays) to confirm that the cells are truly apoptotic. The clonogenic assay is widely used in cancer research to determine the effects of a drug or radiation on proliferating tumor cells by evaluating the survival and proliferation of treated cells. In addition, we have revised the manuscript per the editor’s recommendations and each reviewer’s comments, and we have received the editing services of American Journal Experts.

We attempted in this revision to answer the reviewers’ comments, and we sincerely hope that this revised manuscript is acceptable for publication in BMC Cancer.

Best and kindest regards,

Yong Hyun Jeon, Ph.D.
(Responses to comments)

Editorial Requirements:

Copyediting

After reading through your manuscript, we feel that the quality of written English needs to be improved before the manuscript can be considered further.

We advise you to seek the assistance of a fluent English speaking colleague, or to have a professional editing service correct your language. Please ensure that particular attention is paid to the abstract.

(Answer)

We have received the editing services of American Journal Experts.

Please provide name of ethics committee.

(Answer)

We now describe the name of the ethics committee as the following: “All animal experiment protocols were approved by the Committee for the Handling and Use of Animals, Kyungpook National University”.
Reviewer's report

1. The apoptosis of cells is analyzed by two methods at least, so the authors should give additional necessary experiments to explain whether the cells are true apoptotic cells.

(Answer)

We have performed further experiments to confirm that the cells detected using FACS analysis (with PI and Annexin V staining) are truly apoptotic using a clonogenic assay. The clonogenic assay is widely used in cancer research to determine the effects of a drug or radiation on proliferating tumor cells by evaluating the survival and proliferation of treated cells.

2. The phenotype of cancer cells includes a lot of aspects, so only the change of the level of MHC class I and Fas gene expression cannot be explained to modulate the phenotype of cancer cells.

(Answer)

We agree that further analysis regarding another phenotypic maker such as a tumor antigen is required. Although data concerning intercellular adhesion molecule 1 (ICAM-1) were not presented in this manuscript, we attempted to determine ICAM-1 gene expression levels after treatment with ANT shRNA, I-131 and the combination using FACS analysis. However, we did not observe any changes in ICAM-1 gene expression levels between treated and non-treated cells. Unfortunately, we do not have data for other phenotypes such as tumor antigens. As described above, we analyzed three phenotypic markers, MHC class I, Fas and ICAM-1. As you can see in the references listed below, MHC class I and Fas have been reported to be important..
phenotypic markers that increase the susceptibility of cytotoxic T cells (CTLs) against target cells. Consequently, we focused on these genes in the current study. We trust that these data are acceptable.

[References]


3. According the content of the manuscript, the title is not very suitable.

(Answer)

We have revised the title to “The combination of ANT2 shRNA and hNIS radioiodine gene therapy increases CTL cytotoxic activity through the phenotypic modulation of cancer cells”.

4. The authors performed bioluminescence to visualize the antitumor effect, but the
change of tumor weigh and tumor volume can directly show the antitumor effects. So at the end of treatment, there are not the direct results of tumor weigh and tumor volume. This is why.

(Answer)

In this study, we measured tumor volume using a caliper and weighed the tumor mass. BLI and quantification data were used in the current manuscript because we believe that optical imaging with bioluminescence is sufficient to show the therapeutic intervention of combination therapy. According to the reviewer's recommendation, we have added the tumor volume and weight measurements to supplementary figure 2.

5. In Statistical analysis section, two separate experiments for statistical analysis is not enough, it should need at least three separate experiments.

(Answer)

There was error in the statistical analyses descriptions. All experimental results are derived from at least triplicate experiments. We have replaced the phrase 'two separate experiments' with 'three separate experiments'.

Minor Essential Revisions

1. In method section, the section of apoptosis analysis is too wordy. It need be simplified.

(Answer)

In accordance with the reviewer's comments, we have revised and simplified
the section on apoptosis analysis.

2. In method section, in some place, shRNA is used; in another some place siRNA is used.

(Answer)

There was an error in the description in the methods section. We primarily used shRNA for ANT2 in this study. We have corrected the typographical error.

3. In method section, “Scramble and ANT2 shRNA (100μg/100ul PBS) were intratumorally injected into tumor-bearing mice three times for 3 days…” The authors should explain how many times for one day shRNA was injected. So, it should be revised.

(Answer)

We have revised the description of the methods section for in vivo administration of ANT2 shRNA as the following: 'Scramble and ANT2 shRNA (100 μg/100 ul PBS) were intratumorally injected into tumor-bearing mice once per day for 3 days'

4. In result section, “(HBSS, 50μCi, 300μCi, and 600μCi, 0.0±0.0%, 5.2±1.0%, 10.1±0.5%, and 21.8±1.0%, respectively)…” These descriptions are confusing to me, please consider revising.

(Answer)

We have deleted the detailed explanation for a clearer description in the result
5. Figure 2 (A) is fuzzy, please raise the resolution.

(Answer)

We have increased the resolution of Figure 2A.

6. In discussion section, the authors should mostly explain their own results.

(Answer)

As the reviewer has commented, we have further explained our results regarding the apoptosis analysis and phenotypic modulation analysis in vitro and in vivo in the discussion section. We have explained the results for the in vivo antitumor effects and CTL immune response in the following paragraph of the discussion section, beginning with 'Although we could not evaluate the full spectrum of antitumor immunity.....' Please refer to the revised discussion section.

Quality of written English: Needs some language corrections before being published

(Answer)

We have received the professional editing service of American Journal Experts.