Author's response to reviews


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Author's response to reviews: see over
Responses to comments:

**Reviewer:** Yasunari Nakamoto

**Reviewer's report:**

The authors studied a cancer gene therapy strategy of AFP enhancer/pgk promoter-driven expression of the dominant negative form of the PP2A catalytic subunit α for HCC treatment. Although the results are interesting in thinking the development of novel therapies for liver malignancies, there are some serious concerns with regard to the following points prior to publication.

Comments (Major Compulsory Revisions):

1. Only one AFP-positive cell line HepG2 was used in the current study. To confirm the specificity and effectiveness on AFP-positive HCC cells, other cell lines could be included in the experiments.

   Response: We used another AFP-positive HCC cell line, Hep3B, and the results from this new experiment have been presented in the revised manuscript.

2. To understand the usefulness of cancer gene therapy, the dose-dependent changes of AFP-positive cells (ie. HepG2 cell) by cantharidin and by the recombinant adenovirus (ie. Afpg-DN-PP2Aca) could be evaluated in Figs. 2, 3 and 4.

   Response: The dose-dependent changes by cantharidin are now presented in Fig. 1. The dose-dependent changes by the recombinant adenovirus are presented in Figs. 2 and 4.
Reviewer: Ren-Shyan S Liu

Reviewer's report:

Comments to authors:

1. The authors should provide the results of the expression of luciferase from the following constructs: pGL3-basic-AFpg-Fl, pGL3-basic-pgK-Fl, and pGL3-SV40-Fl in the hepatoma cells, such as HepG2, to demonstrate AFP+ specific transcriptional targeting.

Response: The expression of luciferase was determined by the luciferase reporter gene assay. These results are presented in Fig. 2A.

2. Fig. 2B demonstrates that “pAFpg-Fl” construct (in pGL3 basic backbone) preferentially expressed Fl reporter gene in the AFP+ cells. What is the basal transcriptional activity of the housekeeping gene-pgk? How about the solely pgk promoter-driven Fl activity in the three cell lines?

Response: These results are presented in Fig. 2A.

3. In the experiment of adenovirus treatment of tumor xenograft, the authors measured the tumor size to monitor the therapeutic effect of the constructs: Ad-CMV-DN-PP2Acα, Ad-AFpg-DN-PP2Acα and Ad-AFpg-luciferase. For better demonstration of the effect of gene therapy, bioluminenscence imaging of the tumor xenograft model is mandatory.

Response: It is a very good idea to demonstrate the effect of gene therapy using
bioluminescence imaging. However, the *in vivo* studies were performed quite a long time ago, and we would need to repeat all of these *in vivo* studies again to perform such additional assays. As our time and funds are limited, this additional work would really be too difficult for us. Moreover, the necessary equipment required to perform bioluminescence imaging is not available to us. We are very sorry that we cannot present this result.

4. Page 8, line 5, the mutated site “CCG” should be underlined.

Response: This has been revised.

**Reviewer:** George Chen

**Reviewer's report:**

This manuscript demonstrated that AFP enhancer/pgk promoter-mediated PP2A can specifically kill AFP-positive HCC HepG2 cells in vitro and in a xenograft model. My comments are as follows.

1. The design and experiments of this study is straightforward. However, it lacks the novelty. The use of AFP enhancer/pgk promoter has been previously demonstrated and the cytotoxic property of PP2A is also well known.

Response: The relationship between PP2A and cancer has been widely investigated. PP2A is generally considered to be a tumor suppressor as its inhibition can induce the phosphorylation and activation of substrate kinases that accelerate tumor growth.

Cantharidin, a potent and selective inhibitor of PP2A, is cytotoxic against HCC in
clinical use, which raises the question of why an inhibitor of a tumor suppressor can be used for the treatment of cancer. We have previously proven that the inhibition of PP2A by cantharidin induced the persistent activation of the NF-κB and JNK pathways, which trigger apoptosis and cell cycle arrest. Our studies proved that this inhibition could also be a useful strategy in the treatment of cancer. However, the constitutive expression of PP2A in normal tissues may limit the application of PP2A inhibition. Thus, in the present study, we developed a HCC-specific gene delivery system. Reports regarding the treatment against cancer through the inhibition of PP2A are rare. Importantly, the tissue-specific inhibition of PP2A has not been reported previously.

2. Authors only used a single AFP-positive HCC cell line, HepG2 for this study. The study should be performed in more than one AFP-positive HCC cells to confirm the result.

Response: In the revised manuscript, another AFP-positive HCC cell line, Hep3B, was added.

3. It is wrong to use “infection” in Fig 4C Legend since the Ad was directly injected into the tumor.

Response: This has been revised.

4. Authors did not explain what is PP2Aca.
Response: In the revised manuscript, we have explained this in the second part of the Background.

5. There are a couple of errors throughout the manuscript. For example, Page 14, 1st Para, ‘promoter lead to specific …” should be promoter led to specific..”.

Page 15, 2nd Para, Fig. 3C should be Fig. 4C.

Response: These errors have been revised.