Author’s response to reviews

Title: New anti-cancer chemicals Ertredin and its derivatives, regulate oxidative phosphorylation and glycolysis and suppress sphere formation in vitro and tumor growth in EGFRvIII-transformed cells.

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Version: 1 Date: 13 Apr 2016

Author’s response to reviews:

Dear Reviewer (1):

Thank you very much for providing helpful comments.

We corrected the original manuscript and prepared the revised version as shown in the attached file with the cover letter. Changes are shown in red font.

The main changes to respond to the comments are as follows.
I. We agree that the word “category” is too broad. Thus, we deleted “category” from the title.

II. Based on the comment, we added a few sentences to Page 7 lines 3–8 to explain why low-molecular-weight substances are still needed.

III. As responding to the comments on Page 20 lines 35 -58(figure 1B): We suggest that in NIH3T3/EGFRwt cells, the EGF-EGFR complex is rapidly internalized and degraded within the cell; thus, the downstream survival signal may increase during the early phase, but decrease after 4–5-day culture. In contrast, EGFRvIII is constitutively active without the ligand, and thus the survival signal appears to be transduced in a relatively stable manner. So we changed a few sentences on Page 20 lines 35–58.

IV. As responding to the comments on Figure 2B: We explained the categorizing more precisely and the sentences on Page 21 line 44–page 22 line 18 were modified.

V. As responding to the comments on Page 22 Lines 22-30 and page 27 lines 9-21.

To clearly describe the goal of the screening, we revised Page 22 lines 22–30. The information about the chemical library was shown in Page 8 (Materials section). For explaining another screening source and other few hit samples, we modified the sentences on Page 27 lines 9–26.

VI. According to the reviewer’s suggestion, we revised the sentences on page 23 lines 12–55. The order of two paragraphs was changed.

VII. According to the reviewer’s suggestion, we rephrased the statement on Page 28 lines 38–51,

VIII. To respond to the comments on Page 29 Lines 31-49, we carried out some more experiments but we could not obtain better images than the original one. Thus we provided the better processing images as shown in Additional File 4 (as a new supplementary material) and revised the sentences on Page 29 lines 31–49. Accordingly we changed Fig.8.

Sincerely,

Sonoko Atsumi

Dear Reviewer (2):

Thank you very much for the comment.
We originally wished to use EGFRvIII-expressing natural glioblastoma cells, but found that it is difficult to use the human cell line expressing EGFRvIII to establish an in vitro culture (although a few reports have suggested that is rarely possible). Thus, we used EGFRvIII cDNA-transfected NIH3T3 cell as an in vitro model. Using this model, we could compare the inhibitory potency of substances against EGFRvIII-driven-3D-sphere formation and anchorage-dependent growth of parental normal cells. We are currently studying the effect of Ertredin and its derivatives on human tumors.

These sentences were added to the beginning (Page 20 lines 13-20 in the new manuscript) and end (Page 31 lines 35-38 in the new manuscript) of the Results and Discussion sections.

Sincerely,

Sonoko Atsumi