Author's response to reviews

Title: Nobiletin Suppresses Cell Viability through AKT Pathways in PC-3 and DU-145 Prostate Cancer Cells

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Version: 3
Date: 10 June 2014

Author's response to reviews: see over
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Version: 2 Date: 10 June 2014

Author's response to reviews: see over
Reviewer's report
Title: Nobiletin Inhibits Cell Viability through HIF1- alpha and AKT pathways in PC-3 and DU-145 Prostate Cancer Cells

Version: 1 Date: 30 July 2013

Reviewer: Edy B. Meiyanto

Reviewer's report:

Minor essential revisions as follow:

1. The Title of this paper does not represent the actual result of the research. The title says that Nobiletin inhibits cell viability of both PC-3 and DU-145 cells, but the data shows only PC-3 cells. The word “inhibits” in line 2 and 26 should be replaced by “decrease” or “suppresses” if correspond to viability.

   The title has been changed as the reviewer indicates. The word “inhibits” in line 2 and 26 has been also changed as the reviewer indicates.

2. The abstract of this paper is not completed by the expression of VEGF result. The content presented in the abstract should express the whole result and conclusion of the research.

   The abstract has been revised as the reviewer indicates. The result of VEGF was added to the abstract, the whole result and conclusion of the research were expressed in the abstract too.

3. The antibody for NF-kB used in this experiment should indicate more specific, e.g. p65?

   The antibody for NF-KB is p50, we added it in the manuscript.

4. The presentation of cell viability would be better if completed by presenting of IC-50 value to evaluate the cytotoxic potential of the compound.

   The IC-50 value was shown in Result 3.2.

5. The finding of suppression of VEGF expression is interesting, but actually not directly correspond to the effect on cell viability. Thus, HIF-1A that correspond to VEGF expression also not correspond to the cell viability. The decreasing of cell viability seems to correlate to the decreasing of NF-kB expression. This finding should be elaborated in the discussion more detail and may important to be considered to be included in the title instead of HIF-1A.

   The finding has been elaborated in discussion more detail in paragraph 4 of Discussion and the title has been changed as the reviewer indicates.

6. The authors did not mention the specific aims of the research.
The aim of the research was mentioned in the last paragraph of Background.

7. This paper did not discuss comprehensively about the meaning of cytotoxic effect in relation with underlying mechanism based of the data found in this research, e.g. c-Myc-VEGF-NF-kB and others

The paragraph 4 of the Discussion has been revised as the reviewer indicates. The effects of protein c-Myc, VEGF, NF-kB were expressed. The relations of AKT, NF-kB, Hif-1α and VEGF were also discussed.

8. The conclusion about the inhibitory effect to both of the cells should be refined.

We refined the inhibitory effect to both of the cell lines. We changed word to “suppressed” instead of word “inhibited”.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests
Reviewer's report

Title: Nobiletin Inhibits Cell Viability through HIF1- alpha and AKT pathways in PC-3 and DU-145 Prostate Cancer Cells

Version: 1 Date: 31 July 2013

Reviewer: Akira Murakami

Reviewer's report:

Major Compulsory Revisions
1) This paper by J Chen et al. reports the effects of nobiletin, a natural flavonoid, on proliferation of 2 different prostate cancer cells, and also presented some changes of the expression levels of key proteins in cell growth. Unfortunately, however, no mechanistic link or contribution of those proteins in cell growth regulation by nobiletin except for Fig.9 was demonstrated. The authors should estimate and prove the positive or negative roles of those signaling molecules by using specific inhibitor, siRNA, or expression vectors. Another serious concern is the effective concentrations of this flavonoid. The IC50 values deduced from Fig.2 (~50 uM) would never be achieved in the prostate of the rodents and humans, based on the previous studies on metabolism and absorption of nobiletin.

We studied the link of AKT and Hif-1 α, and the results were also shown in Fig.9. It indicates that nobiletin affects the expression of VEGF, which is the key protein of angiogenesis, through AKT/ Hif-1 α pathway. According to previous reported and our results, we think nobiletin affects cell viability through AKT/NF-kB pathway. The IC50 values may not be achieved in vivo. So it needs in vivo study to warrant the chemoprevention of nobiletin that can be used for suppressing prostate cancer. And we will continue our study about the effect of nobiletin on suppressing prostate cancer.

2) Fig. 7
The authors did not separate the cell lysate into the cytosol and nuclear fractions?

We investigated the NF-kB protein in nucleus of PC-3 and DU-145 cells. The results were shown in Fig.7

Minor Essential Revisions
1) Title
'inhibits cell viability' does not make sense.

The title has been revised. We used word “suppressed” instead of word “inhibits”.
2) Abstract, methods
Gene expression can never be examined by ELISA or WB.
   The abstract has been revised as the reviewer indicates.

Level of interest: An article of insufficient interest to warrant publication in a scientific/medical journal

Quality of written English: Not suitable for publication unless extensively edited

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:
None.
Reviewer's report

Title: Nobiletin Inhibits Cell Viability through HIF1-alpha and AKT pathways in PC-3 and DU-145 Prostate Cancer Cells

Version: 1 Date: 28 August 2013

Reviewer: Liming Zhou

Reviewer's report:

Discretionary Revisions

This study investigated the inhibitory effects of nobiletin on human prostate cancer cells. Data showed that nobiletin inhibited cell viability in both prostate cell lines, with a greater reduction in viability in PC-3 cells. HIF-1α expression and AKT phosphorylation decreased in both cell lines. Nobiletin down-regulated NF-kB expression in PC-3 cells and cMyc expression in DU-145 cells.

Comments:

1. Many flavonoids have chemoprevention and anticancer effects. In this study, the researcher used 0-160umol nobiletin to treat both DU-145 and PC-3 cells, and the inhibitory rate of 160uM nobiletin only 60%. And there is no other experiment has been done to prove the anticancer or chemoprevention effects of nobiletin. Without these considerations and in vivo data makes the results of this study less convincing.

   It indeed needs in vivo study to warrant the chemoprevention of nobiletin that can be used for suppressing prostate cancer. And we will continue our study about the effect of nobility on suppressing prostate cancer. In this paper, we found nobiletin suppressed prostate cells viability by Cell proliferation assay. We also found nobiletin decreased the expression of some key proteins that affect prostate cell viability and angiogenesis. Therefore, nobiletin has potential as a candidate for the chemoprevention of prostate cancer.

2. NF-kb is a protein complex that controls the transcription of DNA. In unstimulated cells, the NF-kB is at inactive form within the cytosol, complexed to an inhibitory IkB-? protein. After various stimuli, ?B-? might be phosphorylated and degraded by releasing the free NF-kB transcription factor. After the free NF-kB translocates into the nucleus, the genes with ?B reporter regions in their promoters may be activated. Thus, western blot of cytosol and nuclear NF-kB should be done to better indicate the involvement of NF-kb in the anti-viability effects of nobiletin.

   We investigated the NF-kB protein in nucleus of PC-3 and DU-145 cells. The results were shown in Fig.7
3. There are some grammars or spelling mistakes in the manuscript, which needs to be revised conscientiously.

   We have revised the grammars and spelling mistakes in the manuscript.