Author's response to reviews

Title: IGF-I activates caspases 3/7, 8 and 9 but does not induce cell death in colorectal cancer cells

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Version: 5 Date: 18 February 2009

Author's response to reviews: see over
Dear Editor,

Thank you for sending us the reviewer’s comments to our paper. We found the reviewer’s constructive comments are helpful. We have carried out some additional work and obtain extra data. According to the new data we have revised the paper and included these new data in the new version of the paper.

Summary of the new version of the paper is as the following:

- Two new figures have been added to figure 3.
- Three new figures have been added to figure 4.
- We included a new figure (figure 5) which included 6 sets of new data.

We would like to use this opportunity to thank reviewers’ comments and suggestion. We believed that the manuscript has been improved and is suitable to be published.

Kind regards

Shiyu Yang

Following is our reply to reviewers’ comments:

Reviewer's report
Title: IGF-I activates caspases 3/7, 8 and 9 but does not induce cell death in colorectal cancer cells
Version: 3 Date: 23 December 2008
Reviewer: Leon A Bach

Reviewer’s report:
The authors have revised the manuscript according to reviewers’ comments but a number of issues remain before the manuscript is acceptable for publication.

MAJOR COMPULSORY REVISIONS
1. Although they provide a direct response to reviewers’ comments, some of the responses have not been incorporated into the manuscript, e.g. the statistical method for multiple comparisons.

The changes have been made according to reviewer’s comments.

2. The authors have measured apoptosis using a single assay. It has not been directly validated against other measures of apoptosis and the
‘validation’ provided shows an extremely modest response to high concentrations of 5FU (<25% increase in apoptosis). The results would be more convincing if a more potent apoptosis inducer was used and apoptosis was demonstrated in various ways.

We used a previously reported cell death assay (Remacle-Bonnet M, et al 2005) to assess cell death after treatment with IGF-I. In order to make double sure this cell death assay is suitable for our experiments we used 5-Fluorouracil (5-FU) to treat three cancer cell lines and then measure the dead cells with this method. We found that the three colorectal cell lines have different sensitivity to the treatments of 5-FU. While HCT116 cells have the most sensitive responses to 5-FU treatment (figure 4 c), HT-29 and SW620 cells only show modest response (figure a and b). These different responses may be due to the divergent genetic background of these cells. HCT116 cell is a p53 wild type of colorectal cancer cell line while HT-29 and SW620 cells contain mutated p53 genes (table 1). Both pre-clinical and clinical studies have shown that disruption of p53 function contributes to 5-FU resistance[26, 27]. Further more this death assay method has been successfully used to determine the apoptotic cell death for HT-29 D4, HCT116 and SW620 cells (Remacle-Bonnet M, et al 2005). We therefore think it is a suitable cell death assessment method for HT-29, SW620 HCT116 cells.

We have changed the contents of manuscript.

3. IGF-I did not stimulate apoptosis despite increasing caspase activity, but the introduction and discussion still largely focus on apoptosis rather than the interesting discrepancy between these observations.

We have added some contents regarding caspase involvement with non-apoptotic function in introduction and discussion.

4. The authors must describe the cell viability assay.

Cell viability assay has been described in new version of manuscript.

5. In their response, the authors allude to a proliferation assay but no data are shown in the manuscript.

Cell proliferation and viability data have been included in the manuscript.

6. It would appear that no isotype-matched control was used for the IGF-IR antibody experiments, raising the possibility that the observed effect was non-specific. This possibility should be mentioned if not rectified.

Thanks to reviewers’ careful thinks. Several extra experiments have been carried out. One of them is a rabbit IgG control experiment within which a
general rabbit IgG was used and it was found that general rabbit IgG was not able to inhibit IGF-I induced caspase activation. This indicates that IGF-1R antibody effect is a specific action. Changes have been made according to the finding.

MINOR COMPULSORY REVISIONS
There continue to be many instances of imprecise language and typographical errors that should be corrected.

English has been checked and changed

The figure legends and text refer to ** as p<0.001, whereas the methods state that this symbol represents p<0.01.

P<0.001 has been changed to p<0.01

DISCRETIONARY REVIEWS

Were caspase 8 and 9 measured in SW and C2 cells and not elevated by IGF-I or were they not measured?

Caspase 8 and 9 was not measured in SW620 and C2C12 cells.

Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Needs some language corrections before being published
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: IGF-I activates caspases 3/7, 8 and 9 but does not induce cell death in colorectal cancer cells
Version: 3 Date: 9 January 2009
Reviewer: Jack Youngren
Reviewer's report:
The authors have done an excellent job of addressing the shortcomings of their original submission. Several minor revisions in the text are necessary, mostly to correct grammatical or technical issues. The changes are as follows:

1. The abstract as well as the introduction (pg 4, line 1) describe the role of IGF-1R in apoptosis as "controversial". Actually, there is little controversy. This should be changed to state that the role of the IGF-1R is not completely understood.
The related contents have been changed according to reviewers’ suggestion and comments.

2. If there is evidence that dysfunction of apoptosis directly contributes to the development of colon cancer, the authors should reference it. Otherwise, this sentence should be removed or qualified.
The sentence has been removed.

3. The final section in the methods (pg 7) validating the cell death assay and referencing figure 4 belongs in the results section.
The changes have been made according to reviewers’ suggestion.

4. In the last sentence of paragraph 2 on page 9 if the results, the word “various” should be changed to “varied” or an equivalent.
“Various” has been replaced by “heterogeneous”.

5. ON page 12, of the discussion, the final three sentences of the first paragraph should be rewritten for clarity. It is not clear what actions of IGF-1 are partially inhibited, or what the conditions are. If the authors are referring to the serum free vs. serum conditions discussed in the final sentence, then the effects of serum are not equivalent to IGF-1 actions. Overall it is not clear what point the authors are trying to make, or why the insulin receptor is invoked.

We try to explain why IGF-I R ab only partially inhibit IGF-I induced caspase activation in some conditions and we find that inhibition effect is more effective in SFM conditions than in SCM conditions, therefore we suggested there may be another growth factor involved with caspase activation. The last three sentences have been changed to make this clear.

6. The first sentence of the seconds paragraph on page 12 should read "there IS EVIDENCE indicating that caspase activation is also involved WITH PROCESSES..."

Changes have been made according to the reviewer’s comments

7. The final sentence of the discussion should be removed. The present studies did not address caspase inhibition. The fact that caspase activation does not lead to an overall increase in cell death is not indicate that inhibition of caspases is without effect. Thus the authors are overly and incorrectly interpreting their results.

The final sentence of the discussion has been removed.
**Level of interest:** An article whose findings are important to those with closely related research interests

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

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

**Declaration of competing interests:**
I hold patents on the use of 2 classes of small molecule IGF-1R inhibitors in the treatment of cancer.

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**Reviewer's report**

**Title:** IGF-I activates caspases 3/7, 8 and 9 but does not induce cell death in colorectal cancer cells

**Version:** 3  **Date:** 9 January 2009

**Reviewer:** Alexandre Arcaro

**Reviewer's report:**

**Major Compulsory Revisions**

1) IGF induces caspase activation, but not apoptosis. What is then the biological relevance of these findings?

   We found exogenous IGF-I increases activities of caspases 3/7, 8 and 9 in three colon cancer cell and one muscle cell lines but IGF-I induced caspase activation does not result in cell death. We don’t know what the biological action of these activated caspase. But we think they may involve with non-apoptotic function. There were studies which found that caspase 3 activation is required for skeletal muscle differentiation and another study which found that terminal differentiation of HT-29 colon cancer cells is tightly linked to caspase activation. It was also proposed that caspases may play roles in cell motility, migration and some cell enucleation. All these have been discussed in the manuscript.

2) If IGF triggers caspase-dependent apoptosis in the cells this should also be assessed by performing cell viability assays (MTT) in the presence or absence of caspase inhibitors (ZVAD).

   Thanks to reviewer’s comment, several experiments have been carried out. One of these experiments is Inhibition of caspases and cell proliferation assay and we found that IGF-I-induced caspase activation has no effect on these cancer cells’ proliferation. Details of these experiments and data have been included in the manuscript.
3) The authors have removed the potentially interesting data on the involvement of PI3K and MAPK in the apoptotic response to IGF. Therefore in its present state the paper lacks mechanistic data describing the molecular events involved in the response to IGF.

Akt and MAPK pathway signalling has been left out in the new version of the paper. We believed these two pathways need more work and we are currently carrying more studies on them now.

**Level of interest:** An article of limited interest  
**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