Author's response to reviews

Title: FHL1C induces apoptosis in Notch1-dependent T-ALL cells through an interaction with RBP-J

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Author's response to reviews: see over
Dear Editors:

Thank you very much for your effort in editing our submitted manuscript entitled “FHL1C induces apoptosis in Notch1-dependent T-ALL cells through an interaction with RBP-J” (MS. No: 1108078689107407) for your consideration of publication in BMC Cancer. We appreciate very much for the reviewers’ thoughtful and constructive comments on our research work reported in this manuscript. We have read these comments very carefully, and performed further experiments according to their suggestions, and make the story reported in our manuscript better. Now I am submitting the revised manuscript for your evaluation. The modified parts in the manuscript are marked by blue color. A point-to-point response to the reviewers’ comments is attached with the revised manuscript. In addition, Dr Si-Yong Huang has substantially taken part in the revision of the manuscript, so we included his name in the author list.

Thank you for your nice consideration and great support.

Best Regards.

Yours Sincerely

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Dear Editor and reviewers:

We appreciate very much for your thoughtful and constructive comments on our research work reported in this manuscript. We have read these comments very carefully, and performed further experiments according to your suggestions, to make the story reported in our manuscript better. Now we make a point-to-point response to your comments, according to the editor, to try our best to answer all the questions you have concerned.

Editorial request:
1) Please clarify who provided informed consent for the use of blood samples from minors.
   Answer: According to the Guideline issued by the Medical Ethics Committee of Fourth Military Medical University, the parents of children under the age of 18 provided informed consent for the use of their blood samples.

2) Please send a copy of the Ethical approval as attachment to an email, including a translation in English.
   Answer: We have included copies of the Ethical approval in both Chinese and English as attachment of our revised paper by email.

3) Please clarify the reason why the muscle biopsy used to obtain RNA: was this patient suffering from a disease, or if not why was the biopsy taken?
   Answer: According to the published paper (Ng EK et al, J Cell Biochem 82:1-10 [2001]), FHL1C is abundantly expressed in testis, skeletal muscle, and heart. In order to obtain mRNA to amplify FHL1C by RT-PCR, skeletal muscle biopsy of a patient suffering from leg trauma was used for RNA extraction. This experiment was performed in accordance with the Guideline issued by Medical Ethics Committee of Fourth Military Medical University.

Reviewer #1:
1. In Page 9 Line 6, it states that “cDNA was diluted appropriately and used for PCR with b-actin as the internal control”. However, according to Figure 1, GAPDH was used as the internal control. Please double check this inconsistency.
   Answer: We are sorry for making this mistake. Indeed, GAPDH was used as internal control in RT-PCR. We have corrected this mistake in the text (Page 9, Paragraph 1, Line 10).

2. The abbreviation “NIC” is not clearly defined. It is better to clearly spell out its full name “Notch1 intracellular domain” when it first appeared.
   Answer: Thank you for your suggestion. Now we have presented the full name of NIC when it first appeared in the text (Page 5, Paragraph 2, Line 11).

3. Please provide information regarding pCMX-VP16-RBP-J. Where did it come from or how was it constructed? What is the sequence characteristic that makes it constitutively active?
This plasmid was kindly provided by Professor Tasuku Honjo (Kyoto University, Japan), which was derived from the plasmid pSG5Flag-RBP-VP16 (Waltzer L et al, Nucleic Acids Res 23:4939-4945 [1995], Kato H et al, Development 124: 4133-4141 [1997]). We are sorry for not providing the detailed source of pCMX-VP16-RBP-J plasmid. Now we have cited the paper published in Development from Prof. Tasuku Honjo's group as Reference 20 (Page 25).

RBP-J fused with the VP16 activation domain leads to the constitutively activation of promoters recognized by RBP-J after being transfected into cells (Waltzer L et al, Nucleic Acids Res 23:4939-4945 [1995]).


Answer: Thank you for your suggestions. We have cited these references in the background section to support our research (Page 5, Paragraph 1, Line 6; Reference 2 and 3).

5. Figure 6E-G is not convincing and supportive to the conclusion. First, the level of b-actin in the nuclear fraction is not equal. Second, the IkB expression in the cytosolic fraction was decreased upon FHL1C overexpression; this change is not consistent with suppressed NK-kB activity. Please provide your interpretation.

Answer: Thank you for your critical comments. (1) We have repeated the experiment shown in Figure 6E, and put more representative pictures in the figure. The results were quantitatively determined from three times of experiments, and the conclusion is not changed. (2) Indeed, the level of IkBα in the cytosolic fraction appeared decreased upon FHL1C overexpression. But statistical analysis of results from three experiments has shown that there is no significant difference between the level of IkB (data not shown). Meanwhile, p50 and c-Rel (two activated components of NF-κB) decreased significantly in the nuclear fraction, suggesting that FHL1C overexpression might down-regulate NF-κB activity. Now we have repeated this experiment with β-actin as a control, and put more representative pictures in the figure to support our conclusion.

Reviewer #2:
Major Compulsory Revisions
1. Patient characteristics are not sufficient, give in addition to table S3 immune phenotype and at least Notch1 mutational status. CR, PR, relapse and death (as given in table S3) are not appropriate categories of outcome because most patients with relapsed T-ALL will eventually die. More appropriate would be alive in CR, death in CR, death after non response, death after relapse, alive in second CR.

Answer: Thank you for your constructive comments. According to your suggestions, we have provided patients’ data in Supplementary Table S3 and made this information more
scientific and complete. As about Notch1 mutational status in these patients, two hot mutation domains, HD and PEST domain of Notch1, were amplified by using Nest-PCR and sequenced. This experiment has been described in Methods (Page 9, Paragraph 1, Line 11-14), and the mutation information of T-ALL patients is presented in Supplementary Table 3 in the revised manuscript.

2. An ensembl transcript ID should be given for FHL1C. The authors need to give sequences for FHL1C RT-PCR primer (not given in table S2). For fig 1 b, the authors should make clear how they distinguish FHL1C from other FHL1 transcripts. In the discussion, the authors seem to use FHL1 and FHL1C as synonyms, but should make clear which splice variant and which protein they refer to.

Answer: Thank you for your critical comments. (1) Transcript ID for FHL1C (007 ENSSSCT00000032876) from ensemble database and the sequences of FHL1C primer have been added in main text (Page 8, Paragraph 1, Line 5-8). (2) We distinguished FHL1C from the other FHL1 transcripts by their size that is reported in the published study (Ng EK et al, J Cell Biochem 82:1-10 [2001]). We add the size of FHL1C and FHL1A in Figure 1A to make the result more clear. (3) FHL1 is a member of the FHL protein family that includes three variants, such as FHL1A, FHL1B and FHL1C. Commonly FHL1 refers to FHL1A; FHL1C is an alternatively spliced variant of FHL1/FHL1A. We have clarified them in the discussion section in the revised manuscript.

Minor Essential Revisions

1. In the abstract, "Current treatments rely on small molecule g-secretase inhibitors..." implies that g-secretase inhibitors are commonly used (which is not true); this should be replaced by "Strategies that employed g-secretase inhibitors to target Notch activation have not been successful".

Answer: Thank you for your good suggestion. Now we change the previous sentences with your suggested sentences (Page 3, Line 2).

2. for Fig 1 b, the proportion of leukemic blasts in patients should be mentioned (or added to table S3). In the discussion, the authors should discuss why they used total PBMC, not more pure T-cells (which would be the appropriate control)

Answer: Thank you for your critical comments. We add the proportion of leukemic blasts in patients in Supplementary Table S3. Certainly, it would have been better to use more pure T cells from PBMC rather than total PBMC. However, it was a little difficult for us to get enough amount of PBMC for T cells purification and RNA extraction. We noticed some groups had used PBMC in their published studies (Haferlach T et al, J Clin Oncol 28: 2529-2537 [2010]; Kohlmann A et al, Clin Chem 54:1705-1715 [2008]), so we used PBMC for the detection of FHL1C and Hes1 expression.

Discretionary Revisions

1. In the abstract, a short sentence should clarify the rationale of looking at FHL1C.

Answer: According to your suggestion, we have clarified the reason for investigating FHL1C in the abstract (Page 3, Line 6).