Author’s response to reviews

Title: FAM168A participates in the development of chronic myeloid leukemia via BCR-ABL1/AKT1/NFκB Pathway

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Author’s response to reviews:

Responses letter

Note: the numbers of the lines refer to these in the revised manuscript.

Editor Comments:

1. In addition to the reviewers comments, please copyedit your manuscript. We suggest that you ask a native English-speaking colleague to help you with this, or to consider using a professional service.

Re: A native English-speaking colleague (a PHD colleague in USA) edited our manuscript. Thank you.
2. The authors showed that TCRP1 interacts with BCR-ABL, however, how about chromosome phenotype and the BCR/ABL fusion of the CML patients included in this study?

Re: In this study, all three patients had chromosome phenotype of 46, XX, t (9:22) (q34: q11) and BCR/ABL1 fusion gene. The clinical features of the patients are shown in Supplementary Table 1. In the line 123 “The clinical features of patients are shown in the Supplementary Table 1” was inserted. Thank you.

3. The authors should include more patients and normal controls in this study. And three independent experiments are required.

Re: Because of the low incidence of chronic myeloid leukemia in children, only 3 samples from children with chronic myeloid leukemia were collected in one year. We are continuing to collect samples from children and adults for our follow-up study.

More than three independent experiments were conducted for real-time PCR, Western blot, and flow cytometry.

In the line 536, 542-543, 567-568, and 586-587 “Data were presented as mean ± SD from three independent experiments.” was inserted.

4. In figure 8, it did not seem that the bands are from a same gel according to the morphology of the bands.

Re: For each sample, we measured FAM168A, AKT1, p-AKT1, IκBα, cyclin B1 and β-Actin. These six target proteins were detected by Western blot on different blotting membranes. Therefore, these bands might appear to be morphologically different. Thank you.

5. Is TCRP1 regulate the apoptosis of the K562 cells?

Re: No evidence shows that FAM168A regulates apoptosis of K562 cells. We deleted a possible regulatory effect of FAM168A on apoptosis in Figure 9. Thank you.
6. In figure 7, qualification is required. In addition, the quality of pictures needs to be proved.

Re: Tumor volume in tumorigenic mice of K562/si-FAM168A group and K562/Control group are added in Figure 7C. The legend for Figure 7 is revised.

In the line 575 “K562 mice” was replaced with “K562/si-FAM168A mice and K562/Control mice”.

In the line 576 “Schematic representation of a mouse in the PBS Control group” was added.

In the line 577-578 “tumors in K562 mice” was replaced with “Schematic representation of a mouse in the K562/Control group.” was added.

In the line 578-579 “C, The tumor volume in K562/si-FAM168A group and K562/Control group.” was added. Thank you.

7. As to the animal study, three independent experiments are required.

Re: Animal experiments were divided into three groups: 562/Control group, K562/siFAM168A group and negative control group. There were 7 mice in each group, so there were already 7 biological repeats in each group. In our future experiments, we will perform three independent experiments. Thank you.

8. It has been proved that AKT is sustained phosphorylated by TRCP1, thus the time curve of AKT phosphorylation is required. And the authors need to prove the effective duration of TRCP1 siRNA, and the relation with the phosphorylation duration of AKT.

Re: It is important to analyze the time curve of AKT phosphorylation. We will perform this analysis in the future. Thank you for your good suggestion.
9. English should be re-checked.

Re: A native English-speaking colleague (a PHD colleague in USA) edited our manuscript. Thank you.

Reviewer #2:

10. P2L9: what is meant by 'therapeutic effects of the disease'? Also, are the authors implying that current TKI strategies, in which the majority of patients presenting in CP will experience deep molecular response, is somehow 'unsatisfactory'? This may need to be qualified.

Re: in the line 28-29 “The pathogenesis of chronic myeloid leukemia (CML) is poorly understood, and therapeutic effects of the disease are still unsatisfactory.” was replaced with “Although the prognosis of chronic myeloid leukemia (CML) has dramatically improved, the pathogenesis of CML remains elusive.” Thank you.

11. P2L25: sentence does not make grammatical sense. P2L53: use HGNC approved gene nomenclature: FAM168A; TCRP1 may be given as a legacy name, but the current approved gene name must be used; also BCR-ABL1.

Re: per your suggestions, in the line 35-37 the sentences “TCRP1 expression was interfered in K562 CML cell line. The expression and phosphorylation of TCRP1 downstream proteins were measured.” was replaced with “FAM168A interference was performed, and the expression and phosphorylation of FAM168A downstream proteins were measured in K562 CML cell line.”

“TCRP1” was replaced with “FAM168A”. BCR-ABL1 is the name of the fusion protein, in which ABL1 gene on chromosome 9 juxtaposed onto the breakpoint cluster region BCR gene on chromosome 22, coding for a hybrid protein. BCR and ABL1 are the currently approved gene names, respectively.

12. P4L17: the sudden switch into discussing childhood leukaemia is potentially confusing; suggest discussing CML in general and breaking metrics into adults and children.

Re: Thank you very much. This paragraph has been revised.
per your suggestion, in the line 61-69 “Leukemia is a malignant clonal disease of hematopoietic stem cells. The leukemia cells proliferate and accumulate in bone marrow and hematopoietic tissues, and infiltrate other tissues/organs, which compromise the normal hematopoietic function. The clinical manifestations of leukemia are anemia, hemorrhage, infection, enlargement of liver, spleen and lymph nodes, as well as bone pain. Childhood leukemia is clinically divided into two categories: acute and chronic. Acute leukemia has acute onset, short duration, and rapid development. In recent years, considerable progress has been made in the treatment of acute leukemia. More than 90% of children with acute leukemia can be cured [1]. Chronic myeloid leukemia (CML) is characterized by myeloid hyperplasia, peripheral blood leukocytosis, and splenomegaly, accounting for 3%-5% of childhood leukemia, with an annual incidence of 1-6 per 100,000 people. The pathogenesis of CML is poorly understood, and therapeutic effects of CML are still unsatisfactory.” was replaced with “Chronic myelogenous leukemia (CML) is a malignant tumor formed by clonal proliferation of bone marrow hematopoietic stem cells. Due to the proliferation and infiltration of cloned leukemia cells in bone marrow and other hematopoietic tissues, normal hematopoietic function is inhibited. The clinical manifestations include anemia, hemorrhage, weight loss, upper abdominal discomfort, and splenomegaly. CML can occur in people of any age, with an annual global incidence of (1.6-2)/100,000 [1]. The incidence of CML increases with age. Although tyrosine kinase inhibitors have made a great breakthrough in the treatment of CML, little is known about the pathogenesis of CML.”

13. P4L47: be consistent in gene/protein nomenclature: BCR-ABL1; also, to be accurate, the Ph chromosome produces a fusion gene, which in turn encodes the BCR-ABL1 fusion protein.

Re: In the manuscript, “BCR-ABL” has been revised to “BCR-ABL1”. Thank you.

In the line 72-73 “Studies have found that the Philadelphia chromosome produces a BCR/ABL fusion protein.” was replaced with “Studies have shown that the Philadelphia chromosome produces a fusion gene, which in turn encodes the BCR-ABL1 fusion protein.” Thank you.

14. P4L53: probably worth noting the p190 (e1a2) variant.

Re: These two variants have sustained tyrosine kinase activity and can promote phosphorylation of downstream signaling proteins. We cannot differentiate the functions of p190 variant from p210 in this study.
In the line 74 the “is called the fusion protein tyrosine kinase p210bcr-abl. p210bcr-abl” was deleted. Thank you.

15. P5L14: 'is discovered'; always use past-tense.

Re:

In the line 80 “was” instead of “is”.

In the line 79 “FAM168A, also known as” was added.

In the line 79 “Genebank accession number: EF363480.1.” was deleted.

16. P5L17: 11q13.4 is a chromosomal location, not a chromosome.

Re: Thank you very much. It has been revised. In the line 81 “q13.4” was deleted.


Re: per your suggestions, in the line 87-88 the sentence “AKT1 expression significantly increased in TCRP1-knockout tongue cancer cell line, and significantly decreased in TCRP1-overexpression tongue cancer cell line. Moreover, NFκB and its downstream gene Bcl-2 were down-regulated or up-regulated correspondingly with the change in AKT1 expression” was replaced with “Moreover, a decrease in AKT1 and NFκB expression was observed in FAM168A-deletion cells.” Thank you.

18. P13L45: what does 's' denote in the data expression? Standard deviation (SD)?

Re: yes, “s” denotes standard deviation. In the line 264 “mean ± SD (standard deviation)” instead of “”. Thank you.
19. P18L15: is it appropriate to use results of experiments to support your argument without showing the data? Either include the data or remove the reference to it.

Re: Thank you very much. In the line 365 the sentence “Our preliminary data showed that TCRP1 expression was elevated in multiple tumors, such as lung cancer, tongue cancer, and ovarian cancer using RT-PCR microarray with tumor specimens and normal controls (data not shown)” was deleted.

20. P19L12: this sentence does not make grammatical sense; also, it seems to make mention of children with CML being included - is this correct?

Re: we agree with you. In the line 385 the sentence “the expression of TCRP1 had increased in PBMCs in children with CML than normal healthy controls. There was no significant difference in AKT1, but the p-AKT1 had significantly increased, and the IκB had decreased.” was deleted.

In the line 382-384 “FAM168A expression in CML patients was higher compared to normal controls. There was no significant difference in AKT1 expression, however, AKT1 phosphorylation and IκB degradation increased.” was added. Thank you.

21. Figure 1: how many technical replicates used? Also, don't use 'average' in technical language, specify median or mean, as appropriate. Also, it needs to be clear what the error bars represent (SD or 95% CI? SEM would not be appropriate).

Re: We collected the data from three independent experiments. In line 536 “Data were presented as mean ± SD from three independent experiments.” was added. Thank you.

22. Figure 2: again, number of technical replicates must be given.

Re: In lane 542-543 the sentence “Data were presented as mean ± SD from three independent experiments.” was added. Thank you.

23. Figure 4: better descriptive legend needed. What does the 'input' lane represent?

Re: per your suggestions, in the line 549-557 the sentence “used for immunoprecipitation. A, Western blot images of protein levels of TCRP1 and BCR-ABL in the immunoprecipitates. B, Western blot images of TCRP1 and AKT1 in immunoprecipitates.” was replaced with “The
lysates were incubated with normal mouse IgG-conjugated agarose (Ig G), anti-FAM168A antibody-conjugated agarose (FAM168A) or anti-ABL1 antibody-conjugated agarose (ABL1). The immunoprecipitants and cell lysates (Input) were electrophoresed and immunoblotted with ABL1 and FAM168A. B, The K562 lysates were incubated with normal mouse IgG-conjugated agarose (Ig G), anti-FAM168A antibody-conjugated agarose (FAM168A) or anti-AKT1 antibody-conjugated agarose (AKT1). The immunoprecipitants and cell lysates (Input) were electrophoresed and immunoblotted with AKT1 and FAM168A.” Thank you.

24. Was the use of paediatric-derived normal PBMC an appropriate control for adult CML?

Re: CML can occur in people of any age. We will collect CML samples from adult patients and normal controls in our future research. Thank you.