Author's response to reviews

Title: BAFF promoted proliferation of human mesangial cells through interaction with BAFF-R

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Version: 3
Date: 5 March 2015

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

Here we re-submitted our revised manuscript "**BAFF promoted proliferation of human mesangial cells through interaction with BAFF-R**" for your consideration in "BMC nephrology" journal. We have conducted some new experiments according to the suggestion of reviewers (Figure 1 and Figure 5). Our answers to each comments of two reviewer are listed below. The grammatical errors have also been corrected in the new version. The revised part of manuscript was written in blue.

We sincerely hope that you can consider our work for your journal and your comments are very welcomed.

With Warm Regards

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**Reviewer:** Kangsheng Li

Q1. P211 The human mesangial cells responded to BAFF treatment significantly only at concentration of 20 ng/ml. Could the author explain the possible reason why higher concentration of BAFF could not stimulate the proliferation of mesangial cells, and could it be used to explain the in vivo...
situation?
Answer: Thanks for the question. Based on our finding that BAFF (20ng/mL) negatively regulated the expression of BAFF-R at presence of 6hrs and 48hrs (Fig. 5), we speculated that larger amount of BAFF (100ng/mL) carry out this action in much shorter time, therefore the proliferative effect of higher concentration of BAFF was not significant. Consistently, Wen et al [1] showed that BAFF promoted the proliferation of T cell at lower but not higher concentration.

Q2. P291 Ref 35 and 36 described the serum levels of BAFF were elevated in IgA nephropathy, which may not be proper to be used as evidence to indicate the LOCAL concentration increasing of BAFF in kidney.
Answer: Thanks for pointing out this. We have deleted one evidence and focus on those confirmed that local BAFF concentration was elevated in kidney of patients with nephritis in the revised version. (P11, L308-311)

Q3. P 302 If possible, the author should find more evidence to demonstrate the BAFF-R could be expressed on mesangial cells in vivo.
Answer: Thanks for the good suggestion. We have tested the BAFF-R antibody (eBioscience,14-9117) for immunofluorescence of kidney biopsy sample, however, it did not work. Due to time limit, we are sorry that we aren't able to provide the expression profile of BAFF-R in human kidney specimen at this moment.

Reviewer2:Xiong-Zhong Ruan

Q1: In Figure 1, the effect of BAFF on mesangial cell proliferation is important. All the cell proliferation experiments in the manuscript were performed at 48h. Perhaps, authors should also do time-courses to optimize the ‘peak time’ for the cell proliferation under action by BAFF. As demonstrated by authors, BAFF decreased BAFF-R expression at 6h (explained as a ‘negative feedback regulation’). Due to the importance of the interaction of BAFF and BAFF-R on the downstream signaling, a short-term experiment for cell proliferation assay should be performed. This will improve the quality of the manuscript significantly.
Answer: Thanks for the good advice. We have tested the time course of HMC proliferation promoted by BAFF from 0hr to 72hr, and we found that BAFF demonstrated its effect from 24hrs to 48hrs (Figure 1D). The 'peak time' for BAFF action on HMC was about 48hrs, as demonstrated by 19.2% percent increase of cell proliferation. After 72hrs, effect of BAFF was less significant, maybe due to exhaustion of nutrition of in vitro culture or the negative
regulation of BAFF-R expression by exogenous BAFF (P9, L240-243).

Q2: In Figure 3, authors demonstrated that human mesangial cells express BAFF-R using real-time PCR. What is the absolute level of BAFF-R in comparison with a well-known mesangial cell marker (positive control?). It is also interesting to know if mesangial cells express BAFF. 
Answer: Thanks for the comment. Since no specific markers of human mesangial cells was identified (P4, L103-107), therefore we still use GAPDH as internal control of BAFF-R in real-time PCR analysis in the revised version. However, mesangial cell have characteristic expression of alpha MSA[2], also can be seen in our study (Figure 5A). The expression level of endogenous BAFF was tested in our study, and it showed that expression of BAFF was quite low as demonstrated in Figure 3A (P9, L262-263).

As shown in Figure 3C, the BAFF-R positive cells are only 8.4%, suggesting that there may be different subgroups (BAFF-R positive and negative) of mesangial cells which may have different functions. In this case, the potential positive downstream effects including cell proliferation, inflammatory response and fibrosis may be diluted if the experiment were carried in whole cell population. Targeting BAFF-R positive cells by dual staining technology using BAFF-R and targeted protein markers of cell cycle protein, inflammation and fibrosis will enlarge/change the biological effects as demonstrated in the manuscript. 
Answer: Thanks for the suggestion. we emphasized that BAFF promoted the proliferation of whole population of human mesangial cell in this study, which try to mimic the general situation in vivo. However, we think that sorting BAFF-R positive mesangial cell will be very helpful to dissect the effect of BAFF on different subsets of mesangial cells for our future study.

Q3: What are the long-term (48h) effects of BAFF on the markers shown in Figure 5?
Answer: Thanks for the suggestion. We have analyzed the long-term effect of BAFF in the revised version. We found that after 48hrs, BAFF still suppressed the expression BAFF-R and Bim (Figure 5) (P11, L296-300).

Q4: Full names should be given for all the abbreviations in the text at the first mention in the text.
Answer: We have given the full names instead of abbreviations for their first time mentioned in the text.