Peer Review File

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Comment 1: The author should provide more context why it is important to investigate the role of REGg in MCL. Is there evidence for differential expression of REGg in MCL, and is this different compared to other lymphoma subtypes?

Reply 1: Thank you for your comment. We added more background to illustrate the importance of the role of REGg in MCL(see Page 4, lines 76-81).

Changes in the text: Despite impressive achievements in targeted drugs, MCL has remained refractory to treatment due to tumoral resistance. The proteasome activator REGγ is implicated in the progression of cancer. As a promoter of cellular growth and a key regulator of several tumor suppressors, many recent studies have linked REGγ overexpression with tumor formation and suggested the REGγ-proteasome as a potential target of new cancer-drug development[4].

Comment 2: In the results section it is described that reducing REGg by shRNA (shR) promotes cell proliferation in JEKO-1, but Figure 1A shows reduced proliferation rate of shR (black solid line) compared to control. Is this a mistake in the Figure?

Reply 2: Thank you for your comment. We are so sorry for the mistake we made in Picture 1A. We have modified the picture and uploaded it again.

Changes in the text: Figure 1A.

Comment 3: The effect of REGg overexpression on cyclin D1 and BCL2 levels as indicated in figure 1F should be quantified to assess whether there is indeed altered expression levels.

Reply 3: Thanks to the reviewer's suggestion, we performed protein quantification of cyclin D1 and BCL2 levels in Figure 1F and confirmed that REG not related to their expression levels. And what we wanted to express originally was also higher expression of REGg promoted Caspase3-PARP-mediated apoptosis and REGg had no effect on cyclin D1 and BCL2 expression (see Page 8, lines 181-182, Supplementary Figure 2).

Changes in the text: In contrast higher expression of REGg promoted Caspase3-PARP-mediated apoptosis (Figure 1F) and REGg had no effect on cyclin D1 and BCL2 expression (Figure S2).

Comment 4: In MCL it has been demonstrated that NF-kB activation promotes cell survival. The phenotype associated with increased Ser536-NF-kB phosphorylation and reduced levels IKB alpha and beta due to REGg knockdown (Figure 3) is in line with NF-kB activation. The effect of REGg knockdown on NF-kB activation in MCL cell line REC-1 is similar to Jeko-1. It will be important to assess whether also the cellular effects of REGg knockdown in the REC-1 cell line on cell proliferation, apoptosis and

cell cycle confirm the data in the Jeko-1 cell line.
Reply 4: Thank you for your comment. We demonstrate that REGg decreased expressions of the three types of IκB subunits and significantly increased levels of NF-κB in REC-1 cells, this is the same as JEKO-1 has a performance (see Page 10, lines 208-211, Figure 3A-B). NF-KB in previous studies (including references 18-19) was confirmed that the activation of it promote the growth of MCL cells. So, we performed the experiment with one cell line, which is one of our experimental design is insufficient, My sincere thanks to the judges for their tips.
Changes in the text: None.

Comment 5: In the discussion, the authors state that REGg is not able to regulate apoptosis in other cell types studied (U937, Namalwa and MEFs), but opposite effects on NF-kB signaling is demonstrated without any functional data on these cellular processes.
Reply 5: We would like to thank the reviewers for pointing out that we have only demonstrated that REGg promotes its expression reduces IκBε and promotes NF-κB signaling by Western blotting in cell types (U937, Namalwa and MEFs), lacking functional cellular assays, and the statement that REGg could not regulate apoptosis in other cell types was lacking of rigor, so we revised the statement (see Page 10, lines 232-233).
Changes in the text: But REGg reduces IκBε and promotes NF-kB signaling in other cell types (human lymphoma U937 cells, Burkitt lymphoma Namalwa cells, and mouse embryonic fibroblasts).

Comment 6: The abstract should provide some extra background information on REGg and Stattic for the less informed reader.
Reply 6: Thank you for your comment. We add some background information on REGg and Stattic in the abstract.(see Page 3, lines 41-44, lines 51-53).
Changes in the text: Mantle cell lymphoma (MCL) is an aggressive B-cell non-Hodgkin lymphoma (NHL). REGγ is important for tumor occurrence and development, but understanding of the specific role of REGγ in MCL is lacking. Cell proliferation, apoptosis, and p-NF-κB, NF-κB, IkB, REGγ, p-STAT3, STAT3, and PSMB5 levels in transfected cells and in transfected cells treated with Stattic, a nonpeptidic small molecule selectively inhibit signal transducer and activator of transcription factor 3 through blocking the function of its SH2 domain, were analyzed using western blotting.

Comment 7: Consistency in the notation of the cell line Jeko-1\JEKO-1 in text and figures. Provide information on the cell lines in Figure 1A-1C, Figure 2.
Reply 7: Thank you for your comment. The JEKO-1 consistency in the text and figures has been modified and cell line information has also been added in Figure 1A-1C, Figure 2.
Changes in the text: Figure 1A-1C, Figure 2.

Comment 8: Line 105/106 mentions digested genomic DNA, but this is probably plasmid/retroviral vector DNA?

Reply 8: Thank you for your comment. we are sorry about our mistake and have modified our text as advised (see Page 5, line 100).

Changes in the text: Plasmid DNA was then circularized with T4 ligase and transformed into DH5α cells to obtain pRevTRE-REGγ. pRevTet-on, pRevTRE, and pRevTRE-REGγ were transfected individually into the viral packaging cell line PT67 using polyethylenimine reagent.

Comment 9: The link between STAT3 and PSMB5 is not well introduced, why do the authors choose this target? Were other targets also examined, which did not provide any differential expression. This information should be included.

Reply 9: Thank you for your comment. We have added a more detailed description of the connection between STAT3 and PSMB5 (see Page 10, lines 219-223).

Changes in the text: PSMB5 is the molecular target of bortezomib, used clinically to ameliorate relapsed multiple myeloma and mantle cell lymphoma. Activated STAT3 levels in cancers may presumably circumvent the effect of bortezomib regimen through up-regulation of PSMB5 protein. These observations suggest that STAT3 modulates PSMB5 expression in MCL[18].