

Peer review file

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Reviewer A

Comment 1: It's an interesting paper about the interaction between Ubiquilin 1, p53, mTOR, and his role in the lung cancer development and progression.

Reply 1: We thank the Reviewer very much for this positive comment!

Reviewer B

In this study, Zhang et al evaluated the role of ubiquitin 1 loss function in the inhibition of apoptosis of A549 adenocarcinoma cells and the mediation of p53 in the process. They demonstrated that knockdown of ubiquitin 1 causes decreased activation of the mTOR/autophagy/p53 pathway leading to inhibition of apoptosis. The study is original but some questions need to be answered to make the study acceptable.

Comment 1: The study was conducted using only one cancer cell line. To reassure this finding it is important to evaluate reproducibility of some of the assays using at least another cancer cell line.

Reply 1: We greatly appreciate the constructive suggestion from the Reviewer. To address this question, we performed the novel experiments in which the effects of UBQLN1 knockdown on cell viability and proliferation were investigated in H358 cells, a non-small cell lung cancer (NSCLC) stable cell line. As shown in Updated Figure S1, loss of UBQLN1 significantly increased cell viability and proliferation in H358 cells. In addition, we also found that UBQLN1 depletion markedly increased the expressions of autophagic markers and reduced p53 protein levels (Updated Figure S2). Moreover, UBQLN1 knockdown-reduced p53 levels were restored by treatment with BFA, an autophagy inhibitor (Updated Figures S2C and S2D). These findings suggest that like in A549 cells, UBQLN1 depletion may enhance cell viability and proliferation in H358 cells through autophagy-dependent downregulation of p53.

Changes in the text: We have indicated the methods for cell culture (Page 4, Lines 13-15), presented the related results (Page 6, Lines 9-11, Lines 18-19; Updated Figure S1 and S2) and figure legends (Page 13, Lines 2-14) in the revised manuscript.

Comment 2: Another point related to the above question is whether the mechanism is only applicable to cancer cells. Similar findings are found if the assays are performed in normal non-cancerous cells?

Reply 2: To address this question, human normal lung epithelial BEAS-2B cells were used to investigate the effects of UBQLN1 knockdown on cell viability and proliferation. As shown in Updated Figure S1, loss of UBQLN1 did not affect cell viability and proliferation in BEAS-2B cells. Combined with the results shown in A549 cells and H358 cells, we could conclude that the promotive effects of UBQLN1 knockdown on cell viability and proliferation is cell type dependent.

Changes in the text: We have indicated the methods for cell culture (Page 4, Lines 13-15), presented the related results (Page 6, Lines 9-11, Lines 18-19; Updated Figure S1) and figure legends (Page 13, Lines 2-6) in the revised manuscript.

Comment 3: Figure 1 (c). The TUNEL assays look like there is no difference between both groups. Improved slide should be presented. The number of n per group in A, B, C, D, F should be shown. How many times each experiment was done. Besides the Bcl-2/Bax ratio, the results of quantification of each Bcl-2 and Bax should be presented separately.

Reply 3: The left panel of Figure 1C is representative images of TUNEL positive cells showing the effects of UBQLN1 depletion on cell apoptosis and the right panel of Figure 1C is its quantificational results based on observation on at least 1000 cells. The apoptotic rate (%) was calculated by dividing the total number of apoptotic cells by the total number of intact cells and multiplying by 100. If calculation was carefully made based on the left panel of Figure 1C, we would find that UBQLN1 loss decreased apoptosis rate from ~35% down to ~10%, which was match with the quantificational results showed on the right panel of Figure 1C. Therefore, we do not think it is necessary to replace Figure 1C, given that statistic difference has been confirmed by the right panel of Figure 1C. In addition, we have indicated the number of n in figure legends and indicated the number of biological replication of each experiments in the section of "2.9 Statistical analysis". Moreover, quantification of protein levels of Bcl-2 and Bax was made in the revised manuscript.

Changes in the text: We have indicated the methods for calculation of apoptosis rate (Page 5, Lines 6-8), sample numbers (section of "Figure Legends"), number of biological replication of each experiments (Page 5, Line 3 from bottom), and quantification of protein levels of Bcl-2 and Bax (Updated Figure 1F; Page 12, Lines 7-8) in the revised manuscript accordingly.

Comment 4: Figure 2. Again, the n in each group and the number of experiments should be given.

Reply 4: We have indicated the number of n in the Figure legends in revised manuscript accordingly.

Changes in the text: Page 12, Line 16.

Comment 5: Figure 4. The images in B should be quantified and evaluate whether there is difference. Again, the number of n in each group and the number of experiments should be given.

Reply 5: We have quantified the changes in Figure 4B and the results were shown in updated Figure 4C. In addition, the number of n has been indicated in the Figure Legends.

Changes in the text: Updated Figure 4C; Page 12, Line 7 from bottom, Line 1 from bottom.

Comment 6: The discussion is a little confusing. To make easier to understand the authors should summarize in a cartoon the conclusion of the study showing the different pathways that were evaluated. By using this cartoon, they will be able to clarify better the relationship between autophagy and apoptosis in the context of cancer.

Reply 6: We have modified the section of discussion and presented a cartoon to summarized the conclusion in the revised manuscript.

Changes in the text: Updated Figure 4F; Page 12, Lines 1-5 from bottom.

Comment 7: The authors suggested that ubiquitin 1 could become a potential target for therapy in cancer. Please explain how could it be applicable?

Reply 7: We have briefly explained this possibility in the revised manuscript.

Changes in the text: Page 8, Lines 1-4 from bottom; Page 9, Line 1.