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AATF/Che-1 acts as a phosphorylation-dependent molecular modulator to repress p53-driven apoptosis

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Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 09 July 2012

Thank you for transferring your manuscript for consideration by the EMBO Journal. As discussed, we sent it for arbitration to two renowned and trusted experts in the field.
You will find their reports appended to this letter and I am pleased to say that both experts share our interest in the study and support publication pending minor revision.

I would like to invite you to submit a revised version of the manuscript, addressing the comments of referee 1:

(i) the referee suggests to replace the QPCR data in SI figs 12-14 with Realtime PCR or to remove the information. We would recommend taking note of this advice as the referee is an expert in this area.

(ii) the referee would like to see the data added in revision in fig 5c on the cell cycle vs. apoptotic target gene Western blots enhanced by including phosphomutants and loss of function for MK2 and/or MRLC3. In our view this additional data would indeed support the conclusions significantly and the experimental suggestions are in principle realistically achievable with reasonable effort. However, we do not want to unduly delay publication of what is a large and detailed dataset. We therefore recommend to include any such data as may be available already or easily added.

We add the following editorial requests:

1) Please edit the manuscript carefully for clarity and length - a terse , clear manuscript attracts more readers in our experience. In particular, we would strongly recommend to consolidate the supplementary information into a smaller number of larger figures (in particular SI figs 1-3; 7-11; 12-14).

2) Please add scale bars to micrographs as appropriate; we encourage a more detailed description of the statistics in figs 5 and 7 (e.g. define n).

3) we strongly encourage the presentation of source data at least for key data panels - that is unprocessed/uncropped versions of the data for Western blots and micrographs. These will be presented as additional data underlying the figures (see previous examples in the journal and our guide to authors).

When preparing your letter of response to the referees' comments, please bear in mind that this may form part of the Review Process File, if you chose to op into this policy (as 97% of our authors), and will therefore be available online to the community. For more details on our Transparent Editorial Process, please visit our website: http://www.nature.com/emboj/about/process.html

As a matter of policy, competing manuscripts published during the revision period will not negatively impact on our assessment of the conceptual advance presented by your study. However, we request that you contact the editor as soon as possible upon publication of any related work, to discuss how to proceed.

Thank you for the opportunity to consider your work for publication. I look forward to your revision. We will not undertake further expert review and will proceed with rapid publication of a suitably revised manuscript.

Yours sincerely,

Editor
The EMBO Journal

REFEREE COMMENTS

Referee #1

I think the paper is interesting, but seems to have exploded during the back and forth at cancer cell. It would now greatly benefit from simplification and some reduction of the data. 8 figures and 14 sup figures are a bit extensive (so are maybe 28 authors).

I would especially like experiments in figure 5C to be redone (maybe with a time-course?) with the AATF wt and mutants used in 5a/b and a MK2 knockdown (this should stop any AATF dependent inhibition of apoptotic targets). To see the outcome of a MRLC3 knockdown would also be interesting- if the phospho switch on AATF is solely to release it from MRLC3, si MRLC3 should mimic phosphorylation (release) and therefore reduce p53 dependent apoptosis. This would not only streamline the paper but prove the suggested model.

Also the semi quantitative PCR shown in some of the supplemental figures are not up to standard anymore (and they clearly have a Realtime PCR); Either lose them or show real-time data.

Finally the manuscript could do with editing to make the text flow more smoothly.

Referee #2

This is a very important study because it identifies another pathway that impacts p53's choice: to kill the cell or to arrest the cell cycle. This is a crucial decision for treating cancers. The authors

responded well to all of the criticisms in the previous review and should be published as is.

1st Revision - authors' response 16 July 2012

Please find below the reviewer´s requests in italics, followed by our responses.

Referee #1

I would especially like experiments in figure 5C to be redone (maybe with a time-course?) with the AATF wt and mutants used in 5a/b and a MK2 knockdown (this should stop any AATF dependent inhibition of apoptotic targets). To see the outcome of a MRLC3 knockdown would also be interesting- if the phospho switch on AATF is solely to release it from MRLC3, si MRLC3 should mimic phosphorylation (release) and therefore reduce p53 dependent apoptosis. This would not only streamline the paper but prove the suggested model.

We thank the reviewer for this thoughtful suggestion. We refer to suppl. Fig. 6, where we show in a functional assay that indeed siRNA mediated knock-down of MRLC3 mimics the anti-apoptotic effects of phospho-dependent AATF release following genotoxic stress. We agree with the reviewer that analyzing expression levels of pro-apoptotic genes in time course experiments by employing AATF-mutants and MRLC3, as well as MK2 knockdown cell lines would make a case to underline the general concept. In order to obtain clear and reproducible data on temporal expression of apoptotic genes, synchronization of cell cycle e.g. with serum starvation is warranted. The existing cell lines, however, were found to be extremely sensitive to starvation and subsequent genotoxic stress precluding this interesting experiment. Establishing an altered protocol for cell cycle synchronization that does not result in profuse sensitivity to genotoxic treatment would take several months. In our opinion the additional impact would not justify the concomitant delay of publication. The effect of AATF and MRLC knockdown on apoptosis and the effect of AATF knockdown on the expression of p53 dependent pro-apoptotic target genes on protein level and in ChIP experiments is strong evidence for a central role of the two genes in the DNA damage response pathway.

Also the semi quantitative PCR shown in some of the supplemental figures are not up to standard anymore (and they clearly have a Realtime PCR); Either lose them or show real-time data.

The data were removed from the manuscript, according to this reviewer´s suggestion.

Finally the manuscript could do with editing to make the text flow more smoothly.

We have focused the manuscript substantially and have reduced its overall length from 72 991 to 64 861 (without references: 57 662) characters. We also rearranged the supplemental figures to cut down from 14 figures to now 7 figures in the revised manuscript.

Referee #2

This is a very important study because it identifies another pathway that impacts p53's choice: to kill the cell or to arrest the cell cycle. This is a crucial decision for treating cancers. The authors responded well to all of the criticisms in the previous review and should be published as is.

We thank this reviewer for this assessment of our work.

Please find below the editorial requests in italics, followed by our responses.

1) Please edit the manuscript carefully for clarity and length - a terse, clear manuscript attracts more readers in our experience. In particular, we would strongly recommend also to consolidate the supplementary information into a smaller number of larger figures (in particular 1-3; 7-11; 12-14).

We appreciate this request and have significantly shortened the manuscript from 72 991 to 64 861 (without references: 57 662) characters. Furthermore, we merged supplemental figures to reduce the number of supplements (Supplemental Figures 1, 2 and 3 to Supplemental Figure 1 and Supplemental Figures 7, 8 and 9 to Supplemental Figures 5).

2) Please add scale bars to micrographs as appropriate; we encourage a slightly more detailed description of the statistics in figs 5 and 7 (e.g. define n).

We added scale bars in all micrographs and described the statistics in Figures 5 and 7 in more detail both in the main text, as well as in the figure captions.

3) We strongly encourage the presentation of source data at least for key data panels - that is unprocessed/uncropped versions of the data for Western blots and micrographs. These will be presented as additional data underlying the figures (see previous examples in the journals and our guide to authors).

In addition to a revised and significantly shortened manuscript, we also submit source data of all key data panels as a separate file accompanying the submitted material.

