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The Cullin 3 substrate adaptor KLHL20 mediates DAPK ubiquitination to control interferon responses

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(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 21 December 2009

Dear Dr. Chen,

Thank you very much for submitting your research manuscript for consideration to The EMBO Journal editorial office. I did originally ask three referees to assess scientific merits and suitability of your paper for publication in our journal. Two scientists have already submitted their overwhelmingly positive reports. I am therefore in the position to make a decision at this point to prevent you and the paper from further unnecessary delay. As you will see both experts very much appreciate the novel mechanism for DAPk regulation. They therefore only request minor additional experiments to test the more general relevance of this in other cellular systems (ref#1) and elucidate potential competition between PML and DAPk for the ubiquitin adaptor KLEIP (ref#3). I kindly ask you to carefully take their comments into account before submitting the ultimate version of your study. I also have to remind you that it is EMBO_J policy to allow a single round of revisions only, which means that the final decision on your work depends on the next and final version of your study.

When you send us your revision, please include a cover letter with an itemised list of all changes made, or your rebuttal, in response to comments from review.
When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process initiative, please visit our website:
http://www.nature.com/emboj/about/process.html

Thank you for the opportunity to consider your work for publication. I look forward to reading the revised manuscript.

Yours sincerely,

Editor
The EMBO Journal

REFEREE REPORTS

Referee #1 (Remarks to the Author):

The authors are to be commended on a well written, thoroughly presented paper in which they elucidate a novel mechanism regulating DAPk protein levels, which involves its interaction with KLEIP, a substrate adaptor protein that links it to the Cul3 E3 ligase complex. They show that this interaction enables the ubiquitination and proteasomal-dependent degradation of DAPk, and that DAPk's functional activity is regulated by KLEIP expression. They furthermore show a physiologically significant stimulus, interferon, that suppresses this mechanism by disruption of the DAPK/KLEIP complex through sequestration of KLEIP to the PML bodies. Their comparison of IFN sensitive and insensitive multiple myeloma cell lines suggests that this may be relevant to the ability of cancer cells to elicit a death response to IFN.

Major points:
1. Is this mechanism limited to as a regulation of the apoptotic function of DAPK, or can it be generalized to other cell types/systems in which DAPk has additional functions- such as autophagy induction in HeLa cells treated with IFN ?

2. The mechanism identified here, involving stabilization of DAPK by IFN, as well as the transcriptional mechanisms that are mentioned in the discussion, are predicted to produce elevated levels of DAPK, however, as DAPK activity is tightly controlled at the level of post-translational modifications, the protein produced should still be inactive. Is their evidence of actual activation of DAPK- for example, through Ca2+/Calmodulin binding, dephosphorylation or phosphorylation of any of the numerous sites preciously identified to regulate DAPK following IFN? Or is the increased in protein levels sufficient to produce DAPK activity that enables death?

3. In Fig 7, the authors demonstrate that the presence or absence of KLEIP-dependent ubiquitination of DAPK affects the status of IFN sensitive and insensitive MM cell lines, by depletion or over-expression of the relevant factors. However, they should verify this more directly by showing that also in these cell lines, DAPk levels are induced in response to IFN in H929 but not XG1 cells, as implied by the apoptotic response, and that this correlates inversely with the degree of the KLEIP/DAPK interaction in these cell types.

Referee #3 (Remarks to the Author):

Review of Lee et al. "The Cullin 3 substrate adaptor KLEIP mediates DAPK ubiquitination to control interferon responses".

Summary of manuscript: Lee and co-workers report on the identification of a BTB-Kelch protein called KLEIP as a binding partner for the Death-Associated Protein Kinase (DAPK). KLEIP is a member of the BTB-Kelch protein family, whose members are generally regarded as substrate adaptors for the Cullin 3 protein. Indeed, the authors demonstrate that KLEIP associates with Cul3 and that a KLEIP targets DAPK for ubiquitin conjugation and subsequent degradation by the proteosome. Subsequent experiments by the authors demonstrate the physiological relevance of the interaction between KLEIP and DAPK. In one set of experiments, the authors demonstrate that treatment of cells with either interferon-alpha or interferon-gamma results the sequesteration of KLEIP into PML bodies, resulting in stabilization of DAPK. In a second set of experiments, the authors demonstrate that sequesteration of KLEIP into PML bodies underlies the sensitivity of a multiple myeloma cell line to apoptosis induced by IFN-gamma treatment

Overall, the experiments are well-performed with the appropriate controls. The findings presented

by the authors significantly extends our understanding of how DAPK is regulated. The work also provides evidence for a new mode of regulation of the Cullin-dependent ubiquitin ligases, in which sequesteration of the substrate adaptor enables the substrate to escape ubiquitin-dependent degradation.

Comments:

1. KLEIP is known by a number of other names. In NCBI, KLEIP is identified as KLHL20. I strongly suggest that KLHL20 name be used throughout this manuscript. The use of a consistent nomenclature will minimize confusion that inevitably arises when one protein has multiple names.

2. PML is markedly upregulated by IFN-gamma (Figure 5). Does PML bind directly to KLEIP? Is there a direct competitive binding relationship between PML and DAPK? In other words, does PML compete with DAPK for binding to KLEIP? Or is there a post-translational modification that alters the affinity of KLEIP for either DAPK or PML? The authors consider if there is a direct competitive relationship, perhaps by adding purified DAPK to anti-KLEIP immunoprecipitates containing bound PML (or purified PML to anti-KLEIP immunoprecipitates containing bound DAPK).

3. There are a few typos and other grammatical errors in the manuscript.

1st Revision - Authors' Response 15 March 2010

We thank the two reviewers for their constructive comments, which serve as the basis for this revised, and, in our view, improved manuscript. Notably, since the name of KLEIP has been changed to KLHL20 in accordance with the reviewer's suggestion, KLHL20 is used throughout this letter.

Referee #1

1. *The reviewer asked whether the mechanism of IFN-regulated, KLHL20-mediated DAPK degradation pathway is limited to the regulation of apoptotic function of DAPK, or it can be generalized to other cell types/systems, such as autophagy induction by IFN.*

Indeed, we presented new data indicating that IFNγ similarly induces KLHL20 relocation to PML-NBs and DAPK stabilization in MCF7 cells undergoing IFN-induced autophagy, (Supplementary Fig. S11 and Fig. 8B). More importantly, we showed that the blockage of KLHL20-mediated DAPK ubiquitination/degradation contributes in part to IFNγ-induced autophagy in MCF7 cells (Fig. 8A, B, and C).

2. *The reviewer raised a point that, even though IFN upregulates DAPK protein, such overproduced protein should still be inactive if it is not dephosphorylated at S308.*

We found that IFN does not increase the amount of S308-phosphorylated DAPK (Supplementary Fig. S11), even though the total level of DAPK is increased significantly. This finding indicates that the overeproduced DAPK can be converted to the active form, and suggests an additional effect of IFN on DAPK, that is, the promotion of S308 dephosphorylation.

3. *The reviewer requested two experiments showing (1) induction of DAPK by IFN in H929 but not in XG1 cells (2) an inverse correlation between DAPK induction and the degree of DAPK/KLHL20 interaction in the two cell types.*

We showed in revised manuscript that (1) IFN triggers a disruption of DAPK-KLHL20 complex in H929 but not in XG1 cells (Fig. 7C), and (2) IFN induces DAPK level in H929 but not in XG1 cells (Fig. 7D).

Referee #2

1. *The reviewer strongly suggests us to change the nomenclature of KLEIP to KLHL20.*

We originally used KLEIP due to two publications which studied the function of this molecule. However, we also agree with the reviewer that utilization of the name in NCBI would minimize confusion. In addition, the name of KLEIP gives little clue on its function and structural identity. We therefore re-named the molecule as KLHL20, and used this nomenclature throughout the manuscript.

2. *The reviewer asked whether there is a direct competitive binding relationship between PML and DAPK.*

We showed in the revised manuscript that DAPK and PML-I (the most abundantly expressed PML isoform) compete for binding KLHL20 both in vivo and in vitro (Fig. 5D and Supplementary Fig. S8). In the in vitro binding assay, baculovirally purified PML-I and KLHL20 were used to bind Flag-KLHL20 isolated from transfected cells. Because a competition between KLHL20 and PML-I was revealed by this study, we believe that these two proteins directly compete for binding KLHL20.

3. *The reviewer indicates the existence of a few typos and grammatical errors in the manuscript.*

The revised manuscript has been edited by a native speaker and therefore these errors should be corrected.

