THE EMBO JOURNAL

Manuscript EMBO-2011-79557

IAPs regulate the plasticity of cell migration by directly targeting Rac1 for degradation

Tripat Kaur Oberoi, Taner Dogan, Jennifer C. Hocking, Rolf-Peter Scholz, Juliane Mooz, Carrie L Anderson, Christiaan Karreman, Dagmar Meyer zu Heringdorf, Gudula Schmidt, Mika Ruonala, Kazuhiko Namikawa, Gregory S Harms, Alejandro Carpy, Boris Macek, Reinhard W. Köster and Krishnaraj Rajalingam

Corresponding author: Krishnaraj Rajalingam, Goethe University Medical School

Review timeline:

Submission date:	20 September 2011
Editorial Decision:	05 October 2011
Revision:	18 October 2011
Pre-Acceptance:	21 October 2011
Accepted:	28 October 2011

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 05 October 2011

Thank you very much for submitting your research manuscript on the role of IAP's as E3-ligases for Rac1 in the regulation of cell shape and motility for consideration to The EMBO Journal editorial office.

Two scientists assessed potential suitability of your study based on the presented manuscript as well as available referee comments from another title. As you can see, the experimental part seems fine as is, the referee's only demand clarification on some statistical presentations (ref#1). Ref#2 recommends careful trimming of the text and correction of some inconsistencies and removal of a few overstatements to present the text as concise and clear as possible.

Despite this, I am very much looking forward to receive an appropriately revised manuscript to your earliest convenience to enable efficient proceedings.

I am very much looking forward to read your minor revisions.

Yours sincerely,

Editor
The EMBO Journal

REFEREE REPORTS:

Referee #1

General Remarks

This study shows that XIAP and cIAP1 bind to Rac1 and promote its ubiquitylation and proteasomal degradation and that lysine 147 of Rac1 is the target of this ubiquitylation. Down-regulation of XIAP and cIAP1 leads to an increase in rac1 protein levels in primary and tumour cells. The authors also show an activated form of rac1 is a better target of XIAP and cIAP1 mediated ubiquitylation. The authors also use a zebrafish model to investigate the effect of XIAP knock-down on a rac dependent phenotype in vivo.

I have only minor issues with the presentation of this study and all are easily dealt with.
-
Specific Remarks

Introduction
Page 4. In addition to the Goyal et al reference the authors should also reference Lisi et al Genetics 2000.

Results section
Page 9. "we then examined if Rac1 is required for the enhanced migration and elongated morphology observed in IAP (space) depleted cells. Consistent with the increase in rac1 levels,....."
Page 10 "We then tested if the phenotypes we observed in tumour cell lines were dependent on rac1 levels"

Discussion
Page 17 " is consistent with the observations made with the DIAP1.."
Figures and Figure Legends

In a couple of the figure legends the error bars were not described eg Fig. 7B and C. "Different types of error bars give quite different information, and so figure legends must make clear what error bars represent." (Cumming et al., 2007)

Figure 4B Seems to be something wrong with the exposure of the GST-Rac1 blots in my pdf. Maybe a Ponceau stain as in Fig 7?

Figure 5A, 5B, 6A, 6C, 7E. The subject of these blots is Rac1 degradation, I think it is therefore more natural for the reader if these panels are put at the top of the figure. The other blots are controls and therefore, in my opinion, are better placed at the bottom.

References
Cumming, G., Fidler, F., and Vaux, D.L. (2007). Error bars in experimental biology. J Cell Biol 177, 7-11.

Referee #2:

This paper by Oberoi et al. investigates the targeting of the small GTPase Rac1, an important modulator of cell shape and motility, by the IAP family of E3 ligases. Importantly, the authors found that the ubiquitylation of Rac1 by cIAP1 and XIAP determines the preferential degradation of the active form of Rac1 by the proteasome, suggesting that this process directly controls cell motility.

This paper contains a wealth of data obtained by in vitro and in vivo experiments, which conclusively demonstrates that IAP E3 ligases directly conjugate ubiquitin to Lys147 of Rac1 and that this phenomenon occurs both in physiological conditions and upon targeting the cycle of

activation/inactivation of Rac1 by CNF and GDI. As such, this data is important and interesting for a broad readership, such that of EMBO Journal.

However, in spite of the overall quality of the primary data, it is surprising to find that their presentation is not always concise, straightforward and free from trivial spelling mistakes and oddities (e.g. zebra fish instead of the more common single word form). To improve these shortcomings, the authors should spend some time in trimming the text to the essentials and removing the repetitions that may have resulted from previous revisions. Streamlining the content of the figures is also highly encouraged to focus the attention of the readers on the most important (and novel) information. Figure 5 is a particular example where a careful selection of the panels and/or shifting some of them to supplemental material could be particularly useful. Additionally, the last scheme in the supplemental material section is more suitable to a review than an original paper. Whilst this work does not involve further experiments, it is of the utmost importance to present the data in the clearest possible way.

This reviewer also invites the authors to eliminate some possible overstatements, which do not add to the paper and make the reading cumbersome in places. To cite a few examples, the discussion is overlong and the reference to cell migration in the title is also not sufficiently qualified and may be removed without altering the final message of this work.

Revision 18 October 2011

We thank both the reviewers for their constructive suggestions and comments and please find our point-by-point response below

Referee #1

General Remarks

This study shows that XIAP and cIAP1 bind to Rac1 and promote its ubiquitylation and proteasomal degradation and that lysine 147 of Rac1 is the target of this ubiquitylation. Down-regulation of XIAP and cIAP1 leads to an increase in rac1 protein levels in primary and tumour cells. The authors also show an activated form of rac1 is a better target of XIAP and cIAP1 mediated ubiquitylation. The authors also use a zebra fish model to investigate the effect of XIAP knock-down on a rac dependent phenotype in vivo.

I have only minor issues with the presentation of this study and all are easily dealt with.

We thank the reviewer for his/her support for publication of these observations in EMBOJ.

-

Specific Remarks

Introduction
Page 4. In addition to the Goyal et al reference the authors should also reference Lisi et al Genetics 2000.

This reference has now been included following the advise of the reviewer.

Results section
Page 9. "we then examined if Rac1 is required for the enhanced migration and elongated morphology observed in IAP (space) depleted cells. Consistent with the increase in rac1 levels,....."

This sentence has been corrected.

Page 10 "We then tested if the phenotypes we observed in tumour cell lines were dependent on rac1 levels"

This sentence has been corrected.

Discussion
Page 17 " is consistent with the observations made with the DIAP1.."

This sentence has been corrected.

Figures and Figure Legends

In a couple of the figure legends the error bars were not described eg Fig. 7B and C. "Different types of error bars give quite different information, and so figure legends must make clear what error bars represent." (Cumming et al., 2007)

Here the error bars represent standard deviations. The text has been corrected accordingly. We have also included an explanation of the error bars following Cumming et al JCB 2007.

Figure 4B Seems to be something wrong with the exposure of the GST-Rac1 blots in my pdf. Maybe a Ponceau stain as in Fig 7?

Yes it is indeed the ponceau staining of the membrane. We have now relabeled the figure.

Figure 5A, 5B, 6A, 6C, 7E. The subject of these blots is Rac1 degradation, I think it is therefore more natural for the reader if these panels are put at the top of the figure. The other blots are controls and therefore, in my opinion, are better placed at the bottom.

We have reorganized the figures as advised by the reviewer. However, we leave it to the editors for the final presentation as the panel may be reorganized to make the best use of available space.

References
Cumming, G., Fidler, F., and Vaux, D.L. (2007). Error bars in experimental biology. J Cell Biol 177, 7-11.

Referee #2:

This paper by Oberoi et al. investigates the targeting of the small GTPase Rac1, an important modulator of cell shape and motility, by the IAP family of E3 ligases. Importantly, the authors found that the ubiquitylation of Rac1 by cIAP1 and XIAP determines the preferential degradation of the active form of Rac1 by the proteasome, suggesting that this process directly controls cell motility.

This paper contains a wealth of data obtained by in vitro and in vivo experiments, which conclusively demonstrates that IAP E3 ligases directly conjugate ubiquitin to Lys147 of Rac1 and that this phenomenon occurs both in physiological conditions and upon targeting the cycle of activation/inactivation of Rac1 by CNF and GDI. As such, this data is important and interesting for a broad readership, such that of EMBO Journal.

We thank the reviewer for supporting the publication of these results in EMBO journal.

However, in spite of the overall quality of the primary data, it is surprising to find that their presentation is not always concise, straightforward and free from trivial spelling mistakes and oddities (e.g. zebra fish instead of the more common single word form). To improve these shortcomings, the authors should spend some time in trimming the text to the essentials and removing the repetitions that may have resulted from previous revisions. Streamlining the content of the figures is also highly encouraged to focus the attention of the readers on the most important (and novel) information. Figure 5 is a particular example where a careful selection of the panels and/or shifting some of them to supplemental material could be particularly useful. Additionally, the last scheme in the supplemental material section is more suitable to a review than an original paper. Whilst this work does not involve further experiments, it is of the utmost importance to present the data in the clearest possible way.

We apologize for the mistakes with the presentation and following reviewers advise, we have redone the manuscript, cutting down the discussion and correcting the spelling mistakes. Figure 5 has been reorganized and some parts of it have now been moved to a new supplementary figure (Figure S6) for space constraints and also to support the flow of the manuscript. The scheme presented as Figure S7 has now been removed from the manuscript.

This reviewer also invites the authors to eliminate some possible overstatements, which do not add to the paper and make the reading cumbersome in places. To cite a few examples, the discussion is overlong and the reference to cell migration in the title is also not sufficiently qualified and may be removed without altering the final message of this work.

We have reorganized the manuscript as advised and the discussion has been trimmed. As the manuscript unveils the role of IAPs in regulating the migration of tumour cells and neuronal progenitor cells we would like to mention migration in the title. However, we have modified the title as "IAPs regulate the plasticity of cell migration by directly targeting Rac1 for degradation" to make it more clear and precise.

Pre-Acceptance 21 October 2011

Thanks a lot for submitting your revised version and the modified text and supplementary information. I like to inform you that I am happy to accept the paper based on the modifications provided.

Furthermore, we now encourage the publication of SOURCE DATA, particularly for electrophoretic gels and blots, with the aim of making primary data more accessible and transparent to the reader. We thus offer the possibility to publish a single PDF file comprising the original, uncropped and unprocessed scans at least for the key data of your paper. These should be labelled with the appropriate figure/panel number, and should have molecular weight markers; further annotation would clearly be useful but is not essential. This PDF will be published online with the article as a supplementary "Source Data" file. Please let me know if you have any questions about this policy, alternatively please check this link for a recent example (http://www.nature.com/emboj/journal/v30/n20/suppinfo/emboj2011298as1.html).

Pending your responses, the editorial office will soon be in touch for official acceptance of your paper.

Please allow me to congratulate to this fine paper.

Yours sincerely,

Editor

The EMBO Journal