THE EMBO JOURNAL

Manuscript EMBO-2010-76243

RNA content in the nucleolus alters p53 acetylation via MYBBP1A

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Review timeline:

Submission date:	14 October 2010
Editorial Decision:	09 November 2010
Revision received:	09 December 2010
Editorial Decision:	22 December 2010
Revision received:	24 December 2010
Accepted:	11 January 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 09 November 2010

Thank you for submitting your manuscript for consideration by The EMBO Journal. It has now been seen by three expert reviewers, whose comments are copied below. As you will see, they all consider your findings on how nucleolar stress induces p53 stabilization and activation potentially interesting, but nevertheless raise a number of issues that would need to satisfactorily addressed before publication may be warranted. A number of these points concern aspects of presentation/interpretation or specific controls, but especially referee 2 also brings up some more substantial experimental questions, for which conclusive answers will be needed.

Should you be able to adequately address these various points in a revised version of the manuscript, then we shall be happy to consider the study further for publication. I should however remind you that it is EMBO Journal policy to allow a single round of major revision only, and that it will thus be important to diligently answer to all the experimental and editorial points raised at this stage. When preparing your letter of response, please also bear in mind that this will form part of the Peer Review Process File, and will therefore be available online to the community in the case of publication (for more details on our Transparent Editorial Process initiative, please visit our website: http://www.nature.com/emboj/about/process.html). Finally, we will also require a brief section specifying the contributions of all individual authors on the paper. In any case, please do not hesitate to get back to us should you need feedback on any issue regarding your revision.

Thank you for the opportunity to consider your work for publication. I look forward to your revision.

Yours sincerely,

Editor
The EMBO Journal

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REFEREE REPORTS:

Referee #1 (Remarks to the Author):

The study by Kuroda et al reveals a role for MYBBP1A in regulating p53 activity by recruiting p300 to acetylate p53 in the nucleoplasm in response to ribosomal stress and this regulation is coupled with the L11 and L5 mediated p53 stabilization. Interestingly, they also found that these two RPs may regulate MYBBP1A shuttling via rRNA. This work offers some mechanistic insight into the ribosomal stress-p53 activation pathway with a substantial amount of neat data. The manuscript is also well written. Addressing the following points could improve their manuscript.

A main point: Could the authors test if knocking down L5 and L11 prevent p53 activation as shown in Figures S3 and 5A independently of MDM2?

Other minor points:

1. It is inappropriate to claim that "...(Figure 5A). These results suggested that MYBBP1A and RPLs functioned on the same pathway that regulated p53 accumulation and acetylation..." in page 15. Literature shows that some ribosomal proteins, including L5 and L11, bind to Mdm2 and stabilize p53 via inhibiting ubiquitination of p53. Also, Figure 5A clearly shows that siL5 or siL11 is more effective on p53 level changing that siMYBBP(compare lane 6 with 7 and 8), which implies that L5 and L11 may also regulate p53 through other pathways, and likely through directly binding to Mdm2.

2. The authors may want to show if the siRNAs in Figure S3 work or not, at least to show si-rpL5, si-rpL11, si-rpL23, and si-rpL26, since they show si-rpL5 and si-rpL11, but not si-rpL23 and si-rpL26, could rescue the activation of p53 after si-TIF-IA.

3. In Figures 5 and S6, the authors show siRNAs for rpL23 and L26. Did they also test these siRNAs in such an experiment? It would be interesting to check if other ribosomal proteins, rather than L11 and L5, are also important for the transportation of MYBBP1A or not. In other words, to test if MYBBP1A transportation is specifically regulated by some, but not other, RPs.

4. Some editorial proofreading is necessary to improve their writing.

Referee #2 (Remarks to the Author):

There is considerable evidence to indicate that the growth-suppressive properties of p53 are activated upon nucleolar stress and that this activation may involve acetylation of p53. In this manuscript, Kuroda et al. used an siRNA approach to identify proteins involved in p53 acetylation / activation upon induction of nucleolar stress. The authors report that upon nucleolar stress, MYBBP1A translocates from the nucleoli to the nucleoplasm and activates p53 by stabilizing the interaction of p53 with the acetyltransferase p300. Furthermore, evidence is provided that the continuous presence of the ribosomal proteins L5 and L11 are required for MYBBP1A translocation. The notion that MYBBP1A regulates p53 acetylation / activation via p300 is potentially interesting. However, although the authors have performed a considerable number of experiments to support the main conclusions, there are number of issues that need to be addressed before the manuscript should be considered for publication;

1. All of the experiments with endogenous proteins were performed with MCF-7 cells. To provide

evidence that the data are of general significance (and not a peculiarity of MCF-7 cells), key experiments should be repeated in additional cell lines expressing wild-type p53 (e.g. U2OS, RKO).

2. Fig. 1F: I agree with the authors that rescue experiments are the most convincing approach to show that RNAi effects observed are not due to off-target effects. However, it did not become clear to me why the authors decided to perform the rescue experiment in H1299 cells rather than in MCF-7 cells (as all of the other data were obtained in MCF-7 cells).

3. Fig. 3E: Firstly, is coexpression of p300 indeed required for p53-induced expression of p21? Secondly, is the p53-KR mutant still active as transcriptional transactivator (if not, the experiment is meaningless)? Thirdly, it should be shown that the p53-KR mutant still binds to MYBBP1A (Suppl. Info).

4. Fig. 3F: It is shown that knockdown of MYBBP1A expression rescues the cytotoxic effect of TIF-IA knockdown. Are these effects indeed dependent on the presence of wild-type p53 (e.g. what happens to H1299 cells upon knockdown of TIF-IA expression?)?

5. Throughout the manuscript, the authors are referring to the importance of the C-terminal lysine residues of p53 for acetylation and ubiquitylation/degradation. However, most of the respective published data were obtained in ectopic expression experiments studying p53 mutants (i.e. replacement of respective lysine residues by arginine). Notably, in a mouse model, the C-terminal lysine residues of p53 are required neither for stability regulation nor for transactivation (Krummel et al., 2005, PNAS 102, pp.10188). The authors should consider these data in the introduction and when discussing their results.

6. The introduction is overly long and can be significantly shortened.

Referee #3 (Remarks to the Author):

Over the past decade, the relationships among cellular stress, nucleolar disruption and p53 stability have become increasingly important. The work presented in this manuscript represents a systematic analysis of proteins that translocate from the nucleolus to the nucleoplasm under stress conditions to potentially stabilize p53. This was a major undertaking that identified several pieces of the puzzle. The importance of these observations is that the mode of action of MYBB1A is different from previously-discovered proteins that are released from the nucleolus under stress conditions. The work also shows that p53 stabilization can occur with acetylation and not phosphorylation. In addition, PRL 5 and RPL 11 were shown to be essential for rRNA transport and consequent p53 activation. These are novel findings that warrant publication in a high profile journal.

With the exception of a few points indicated below the manuscript is very well written and the results appear to be supported by the data. The work should be of major interest to those researchers involved with the nucleolus and the stress response. Although I am not fond of the large numbers of supplementary figures that are referred to in the text, this issue should be left to the editor's discretion. The following minor points should be addressed before publication is considered.

1. On p. 9 it is stated that nearly 300 proteins translocate from the nucleolus following nucleolar disruption. In the next sentence it is implied that siRNAs were generated for all of these. However, Table S1 lists only 107 of them. Please clarify this in the text.
2. Fig. 2D. A statement on p. 12 says that the association of p53 with p300 was enhanced by MYBBP1A expression in the presence of ADR. We see more p53,but not p300. Shouldn't we see more p300 also if the association is enhanced? Legend. Spell out adriamycin. Also, HAT is mentioned in the legend, but is not indicated in the figure.
3. P. 13 and Fig. 3E. The paragraph describing these experiments is technically correct, but seems in this reviewer's mind, difficult to understand. Try to enhance the readability of this section.
4. P. 14, top. There are really too many supplementary figures. In this case, the results are essentially confirmatory and they could simply be mentioned as data not shown.
5. Fig. 4A. Do NPM and EBP2 run together? If so, mention this in the legend.
6. P. 14, bottom and Fig. 4B. Although MYBBP1A is released by RNase, one cannot rule out that it is only indirectly bound to RNA through other proteins. It could be part of a larger complex that is

released by RNase. Please indicate that this is a possibility.
7. P. 17 and Fig. 6C. The RNA content of the nucleoli has increased and the nucleoli are enlarged in the siRP-treated cells. Please include this observation in your discussion of the results on p. 17.

1st Revision - authors' response 09 December 2010

Point-by-point responses for reviewer #1

We are grateful to the reviewer for the critical comments and useful suggestions that have helped us improve our paper. As indicated in the responses that follow, we have taken these comments and suggestions into account in the revised version of the paper.

***** Referee #1 *****
A main point:
Could the authors test if knocking down L5 and L11 prevent p53 activation as shown in Figures S3 and 5A independently of MDM2?

To address the reviewer's comment, we performed TIF-IA knockdown together with HDM2 and RPL5/11 (Revised version: Figure 5B). In summary, the results showed that RPL5/11 regulated p53 protein levels and acetylation via two pathways, an HDM2-dependent and an HDM2-independent pathway. As shown in Figure 5B, knockdown (KD) of TIF-IA enhanced the accumulation and acetylation of p53 (lane 1 in the new Figure 5B; No. 1 in the Figure shown below). This enhancement by TIF-IA KD was abrogated by RPL5/11 KD (lanes 2 and 3 in the new Figure 5B; No. 2 in the Figure shown below). To examine the contribution of HDM2 to the regulation of p53 by RPL5/11, HDM2 was further knocked down with TIF-IA and RPL5/11. The results showed that HDM2 KD partially recovered the reductions in p53 protein and acetylation levels caused by RPL5/11 KD (lanes 5 and 6 in the new Figure 5B; No. 4 in the Figure shown below). These results indicated the involvement of HDM2 in p53 regulation by RPL5/11. However, the partial HDM2-mediated recovery of reductions in p53 accumulation and acetylation induced by RPL5/11 KD suggested the presence of an HDM2-independent pathway in p53 activation. These results, together with our other data and previous reports, indicate that RPL5 and RPL11 activate p53 by two mechanisms: inhibition of HDM2 (an HDM2-dependent pathway), and induction of MYBBP1A translocation from the nucleolus to the nucleoplasm (an HDM2-independent pathway).

We have included this explanation in the text. (Revised version: text page 13, line 24 through text page 14, line 18)

Other minor points
1. It is inappropriate to claim that "...(Figure 5A). These results suggested that MYBBP1A and RPLs functioned on the same pathway regulated p53 accumulation and acetylationÖ" in page 15. Literature shows that some ribosomal proteins, including L5 and L11, bind to Mdm2 and stabilize p53 via inhibiting ubiquitination of p53. Also, Figure 5A clearly shows that siL5 or siL11 is more effective on p53 level changing that siMYBBP (compare lane 6 with 7 and 8), which implies that L5 and L11 may also regulate p53 through other pathways, and likely through directly binding to Mdm2.

According to the reviewer's suggestion, we performed additional experiments (Revised version: Figure 5B). As suggested by the reviewer, our results showed that there are two pathways for p53 regulation by RPL5/11. One pathway is HDM2-dependent, and the other pathway is MYBBP1A-dependent (HDM2-independent).

2. The authors may want to show if the siRNAs in Figure S3 work or not, at least to show si-rpL5, si-rpL11, si-rpL23, and si-rpL26, since they show si-rpL5 and si-rpL11, but not si-rpL23, and si-rpL26, could rescue the activation of p53 after si-TIF-IA.

We have evaluated the knockdown efficiency of RPL5, RPL11, RPL23, and RPL26 siRNA (Revised version: Supplementary Figure S4B). RT-qPCR analysis revealed that mRNAs of these RPLs were knocked down by >90%.

3. In Figure 5 and S6, the authors show siRNAs for rpL23 and L26. Did they also test these siRNAs in such an experiment? It would be interesting to check if other ribosomal proteins, rather than L11 and L5, are also important for the transportation of MYBBP1A or not. In other words, to test if MYBBP1A transportation is specifically regulated by some, but not other, RPs.

According to the reviewer's suggestion, we have examined the effects of RPL23 or RPL26 siRNA on MYBBP1A transport (Revised version: Supplementary Figure S11). Immunostaining showed that knockdown of RPL23 or RPL26 could not inhibit the translocation of MYBBP1A and NPM from the nucleolus to the nucleoplasm induced by TIF-IA knockdown, ActD, or ADR treatment. These results suggest that MYBBP1A transport is specifically regulated by RPL5 and RPL11.

We have added the following sentence to the text. (Revised version: text page 15, lines 3-5)

4. Some editorial proofreading is necessary to improve their writing.

We will entrust the editorial proofreading to the editor.

Point-by-point responses for reviewer #2

We are grateful to the reviewer for the critical comments and useful suggestions that have helped us improve our paper. As indicated in the responses that follow, we have taken these comments and suggestions into account in the revised version of the paper.

****** Referee #2 ******
1. All of the experiments with endogenous proteins were performed with MCF-7 cells. To provide evidence that the data are of general significance (and not a peculiarity of MCF-7 cells), key experiments should be repeated in additional cell lines expressing wild-type p53 (e.g. U2OS, RKO).

To address the reviewer's comment, we repeated key experiments using LNCaP human prostate cancer cells, which also express wild-type p53 (Revised version: Supplementary Figure S3 and S9). We obtained similar results for all experiments.

2. Fig. 1F: I agree with the authors that rescue experiments are the most convincing approach to show that RNAi effects observed are not due to off-target effects. However, it did not become clear to me why the authors decided to perform the rescue experiment in H1299 cells rather than in MCF-7 cells (as all of the other data were obtained in MCF-7 cells).

As the reviewer suggested, we performed the rescue experiment to eliminate the possibility of off-target effects. To determine the effects of MYBBP1A on p53 acetylation, we had to compare the acetylation levels of p53 among cells expressing the same levels of p53 protein. In MCF-7 cells, knockdown of TIF-IA and/or MYBBP1A altered endogenous p53 protein levels. In contrast, because H1299 cells do not express endogenous p53 protein, it was possible to exogenously express an equal quantity of p53 protein, even if TIF-IA and/or MYBBP1A were knocked down. Therefore, we exogenously expressed equal quantities of p53 and compared these acetylation levels in H1299 cells.

3. Fig. 3E: Firstly, is coexpression of p300 indeed required for p53-induced expression of p21? Secondaly, is the p53-KR mutant still activate as transcriptional transactivator (if not, the experiment is meaningless)? Thirdly, it should be shown that the p53-KR mutant still binds to MYBBP1A (Suppl. Info).

Response to the first question: The exogenous expression of p53 alone only slightly induced p21 because the amount of endogenously expressed p300 protein was insufficient for acetylation of the exogenously expressed p53. Therefore, it was necessary to coexpress p300 with p53.

Response to the second question: To answer the reviewer's question, we examined the expression of another p53-target gene, PUMA (Revised version: Figure 3E). The expression of PUMA was induced by exogenous expression of the p53-KR(p300) mutant. The p53-KR(p300) mutant was found to retain transcriptional activity.

Response to the third question: In response to the reviewer's suggestion, we show evidence for the interaction between p53-KR(p300) and MYBBP1A in the new Supplementary Figure S7.

4. Fig. 3F: It is shown that knockdown of MYBBP1A expression rescues the cytotoxic effect of TIF-IA knockdown. Are these effects indeed dependent on the presence of wild-type p53 (e.g. what happens to H1299 cells upon knockdown of TIF-IA expression?)?

To answer the reviewer's question, we performed additional experiments using H1299 cells (Revised version: Supplementary Figure S8A). Our data revealed that TIF-IA knockdown could not cause apoptosis in H1299 cells. We observed the same results using p53-knocked-down MCF-7 cells (Revised version: Supplementary Figure S8B). These results indicated that the cytotoxic effects of TIF-IA knockdown were dependent on the presence of wild-type p53.

We have added the following sentence to the text. (Revised version: text page 12, lines 8-9)

5. Throughout the manuscript, the authors are referring to the importance of the C-terminal lysine residues of p53 for acetylation and ubiquitination/degradation. However, most of the respective published data were obtained in ectopic expression experiments studying p53 mutants (i.e. replacement of respective lysine residues by arginine). Notably, in a mouse model, the C-terminal lysine residues of p53 are required neither for stability regulation nor for transactivation (Krummel et al., 2005, PNAS 102, pp. 10188). The authors should consider these data in the introduction and when discussing their results.

We have included references to this manuscript in the introduction and discussion sections and discussed the discrepancies between their results and our data. (Revised version: text page 4, lines 16-19 and text page 18, lines 4-12)

6. The introduction is overly long and can be significantly shortened.

According to the reviewer's suggestion, we have significantly shortened the introduction.

Point-by-point responses for reviewer #3

We are grateful to the reviewer for the critical comments and useful suggestions that have helped us improve our paper. As indicated in the responses that follow, we have taken these comments and suggestions into account in the revised version of the paper.

***** Referee #3 *****

1. On p. 9 it is stated that nearly 300 proteins translocate from the nucleolus following nucleolar disruption. In the next sentence it is implied that siRNAs were generated for all of these. However, Table S1 lists only 107 of them. Please clarify this in the text.

According to the reviewer's comment, we have added an explanation in the text (Revised version: text page 7, lines 14-16) as follows; "Among these 300 proteins, we excluded 200 proteins based on their known functions and selected 107 candidates for investigation."

2. Fig. 2D. A statement on p. 12 says that the association of p53 with p300 was enhanced by MYBBP1A expression in the presence of ADR. We see more p53, but not p300. Shouldn't we see more p300 also if the association is enhanced? Legend. Spell out adriamycin. Also, HAT is mentioned in the legend, but is not indicated in the figure.

In this experiment, an equal amount of FLAG-p300 was exogenously expressed and immunoprecipitated using a FLAG-antibody. Therefore, it is reasonable that an equal amount of

FLAG-p300 was detected among the immunoprecipitants (Revised version: Figure 2D, lanes 11 and 12). On the other hand, higher quantities of p53 were co-precipitated with FLAG-p300 proteins from the lysates of cells that overexpressed MYBBP1A than from the control cells (Revised version: Figure 2D, compare lane 12 with 11). These results indicated that the interaction between p300 and p53 was enhanced by MYBBP1A expression.

At the reviewer's suggestion, we spelled out adriamycin in the figure legend and changed "Acetyltransferase" to "HAT" in the Figure.

3. P.13 and Fig. 3E. The paragraph describind these experiments is technically correct, but seems in this reviewer's mind, difficult to understand. Try to enhance the readability of this section.

At the reviewer's suggestion, we have improved the explanation of these experiments. This sentence was proofread by native speakers. (Revised version: text page 11, line 13 through text page 12, line 4)

4. P.14, top. There are really too many supplementary figures. In this case, the results are essentially confirmatory and they could simply be mentioned as data not shown.

We will follow the editor's guidance and suggestions with respect to this issue.

5. Fig. 4A. Do NPM and EBP2 run together? If so, mention this in the legend.

NPM and EBP2 were identified in the same protein band. Because the molecular weights of NPM and EBP2 are similar, these two proteins ran together under the conditions of our experiments. This has been noted in the figure legend. (Revised version: Figure 4A legend)

6. P.14, bottom and Fig. 4B. Although MYBBP1A is released by RNase, one cannot rule out that it is only indirectly bound to RNA through other proteins. It could be part of a larger complex that is released by RNase. Please indicate that this is a possibility.

We agree with the reviewer's comment. We could not rule out that MYBBP1A indirectly bound to RNA through other proteins; MYBBP1A was found to interact with RNA-binding proteins (Figure 4A) and did not contain obvious candidate RNA-binding domains. According to the reviewer's comment, we have indicated this possibility in the text. (Revised version: text page 13, lines 4-10)

7. P. 17 and Fig. 6C. The RNA content of the nucleoli has increased and the nucleoli are enlarged in the siRP-treated cells. Please include this observation in your discussion of the results on p. 17.

According to the reviewer's comment, we have added the following sentence to the text (Revised version: text page 15, lines 16-19): "We also found that the nucleoli in RPL5 or RPL11 siRNA-treated cells appeared to be larger than those in the control siRNA-treated cells. This result may reflect an increase in nucleolar RNA content in RPL5 or RPL11-depleted cells (Figure 6A)."

2nd Editorial Decision

Thank you for response and providing the requested additional information.

On conducting the routine pre-acceptance CrossCheck procedure with your manuscript, I am afraid it has come to our attention that there are some major issues after all that currently still preclude publication and will therefore need to be addressed through an additional round of revision.

These problems concern several passages in the manuscript text, which are highly similar to several publications. In particular, this concerns the whole 'Methods' paragraph on 'Chromatin Immunoprecipitation(ChIP)', whose passages can be found in almost identical form in three

publications by the group of Carsten Carlberg (Saavalainen et al JBC 2005, Sinkkonen et al NAR 205, Saramaki et al NAR 2006 - none of which are cited). Of course I appreciate that Method sections may not be held to the same standards as introduction, results or discussion sections, and that it is difficult to fully rewrite them every time. However I hope you understand that the paragraphs in the current form cannot be accepted, as you might be held responsible for plagiarism if the paper was to be published in the present form. Furthermore, I strongly feel that if a method has been adapted in identical form from previous studies, then these studies should also receive mention and be included as references. I therefore need to ask you to address these issues by rewriting the respective section and introducing relevant citations where appropriate.

Another, related concern is the high similarity of several passages in the manuscript with two abstracts from your group published as conference proceedings in the European Journal of Cancer earlier this year. Clearly these abstracts are your own intellectual property, and it is also our editorial policy to not consider conference proceedings as preempting the novelty of a full paper. However also here, in light of the current research integrity standards the high text identities could be considered as a form of self-plagiarism were they to be published in this form, so again I will need to kindly ask you to slightly re-phrase the respective paragraphs.

The last outstanding issue pertains to the careful editorial proofreading that had been initially requested by the referees: this is an author's responsibility which I am afraid cannot be shifted to the editors, as we neither have the required copy editor staff nor the profound insight into the respective subject matter and the message that an author wants to convey. Therefore, please do carefully proofread the paper before resubmitting, maybe soliciting the help of a colleague well-versed in the English language.

In conclusion, I am herewith returning the manuscript to you for an exceptional extra round of text revision, pending on whose satisfaction we will then hopefully be able to proceed with acceptance and publication of the paper. Should you have any questions in this regard, please do not hesitate to get back to us.

Yours sincerely,
Editor
The EMBO Journal

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REFEREE REPORTS:

Referee #1 (Remarks to the Author):

The authors have addressed all the points as raised by previous reviewers. In terms of editorial issues, they specifically mentioned to leave them to the journal editor. I think as long as the language has no major issues, this manuscript is ready for publication. This study builds a potential link between ribosomal stress and p53 acetylation, which have been observed previously but not tightly associated previously by any other labs as done in this work. Thus, I would highly regard this as a quite novel and important finding.

Although I am a bit skeptical about the partial effect of knockdown of MDM2 on the p53 level and acetylation, I am willing to accept it for EMBO, as they have done tremendous amounts of work in this single manuscript.

2nd Revision - authors' response 24 December 2010

We would like to resubmit the revised version of our manuscript (Revise # EMBOJ-2010-76243R) entitled "RNA content in the nucleolus alters p53 acetylation via MYBBP1A". We have addressed the comments cited by editors below.

Editor's comments
These problems concern several passages in the manuscript text, which are highly similar to several publications. In particular, this concerns the whole 'Methods' paragraph on 'Chromatin Immunoprecipitation(ChIP)', whose passages can be found in almost identical form in three publications by the group of Carsten Carlberg (Saavalainen et al JBC 2005, Sinkkonen et al NAR 205, Saramaki et al NAR 2006 - none of which are cited). Of course I appreciate that Method sections may not be held to the same standards as introduction, results or discussion sections, and that it is difficult to fully rewrite them every time. However I hope you understand that the paragraphs in the current form cannot be accepted, as you might be held responsible for plagiarism if the paper was to be published in the present form. Furthermore, I strongly feel that if a method has been adapted in identical form from previous studies, then these studies should also receive mention and be included as references. I therefore need to ask you to address these issues by rewriting the respective section and introducing relevant citations where appropriate.

We rewrote the paragraph on 'Chromatin Immunoprecipitation(ChIP)' and cited the references which you suggested. (Revised version: text page 23, line 20 through text page 24, line 3)

Another, related concern is the high similarity of several passages in the manuscript with two abstracts from your group published as conference proceedings in the European Journal of Cancer earlier this year. Clearly these abstracts are your own intellectual property, and it is also our editorial policy to not consider conference proceedings as preempting the novelty of a full paper. However also here, in light of the current research integrity standards the high text identities could be considered as a form of self-plagiarism were they to be published in this form, so again I will need to kindly ask you to slightly re-phrase the respective paragraphs.

In response to your comments, we re-phrased the respective paragraphs in the abstract. (Revised version: text page 2, lines 1-17)

The last outstanding issue pertains to the careful editorial proofreading that had been initially requested by the referees: this is an author's responsibility which I am afraid cannot be shifted to the editors, as we neither have the required copy editor staff nor the profound insight into the respective subject matter and the message that an author wants to convey. Therefore, please do carefully proofread the paper before resubmitting, maybe soliciting the help of a colleague well-versed in the English language.

According to the referees' request, the manuscript has been proofread by a native speaker.

We are most grateful to you for helpful comments. We hope our responses and revisions have made this paper acceptable for publication in The EMBO Journal.

Acceptance letter 11 January 2011

Thank you for submitting your re-revised manuscript for our consideration. Please excuse the delayed response, but I have been away from the office for the past two weeks. I have now had a chance to look through it and to assess the changes made in response to my previous decision letter, and I am happy to inform you that there are no further objections towards publication in The EMBO Journal.

You shall receive a formal letter of acceptance shortly.

Yours sincerely,

Editor
The EMBO Journal

