Post-transcriptional regulation by the exosome complex is required for cell survival and forebrain development via repression of P53 signaling

Pauline Antonie Ulmke, Yuanbin Xie, Godwin Sokpor, Linh Pham, Orr Shomroni, Tea Berulava, Joachim Rosenbusch, Uttiya Basu, Andre Fischer, Huu Phuc Nguyen, Jochen F. Staiger and Tran Tuoc
DOI: 10.1242/dev.188276

Editor: Paola Arlotta

Review timeline

Original submission:	16 January 2020
Editorial decision:	24 February 2020
First revision received:	24 August 2020
Editorial decision:	19 October 2020
Second revision received:	17 December 2020
Accepted:	29 December 2020

Original submission

First decision letter

MS ID#: DEVELOP/2020/188276

MS TITLE: Post-transcription regulation by the exosome complex is required for cell survival and forebrain development by repressing P53 signaling

AUTHORS: Pauline Antonie Ulmke, Yuanbin Xie, Linh Pham, Orr Shomroni, Joachim Rosenbusch, Tea Berulava, Andre Fischer, Uttiya Basu, Huu Phuc Nguyen, Jochen Staiger and Tran Tuoc

I have now received all the referees' reports on the above manuscript, and have reached a decision. The referees' comments are appended below, or you can access them online: please go to BenchPress and click on the 'Manuscripts with Decisions' queue in the Author Area.

As you will see, the referees express considerable interest in your work, but have some significant criticisms and recommend a substantial revision of your manuscript before we can consider publication. If you are able to revise the manuscript along the lines suggested, which may involve further experiments, I will be happy receive a revised version of the manuscript. Your revised paper will be re-reviewed by one or more of the original referees, and acceptance of your manuscript will depend on your addressing satisfactorily the reviewers' major concerns. Please also note that Development will normally permit only one round of major revision.

Please attend to all of the reviewers' comments and ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost in PDF conversion. I should be grateful if you would also provide a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

© 2021. Published by The Company of Biologists under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/).

Reviewer 1

Advance summary and potential significance to field

Ulmke and colleagues present a work describing the effects of Exosc10 functional inactivation on mouse cortical development by the analysis of cKO_Emx1-Cre mice. Using RIP-seq analysis and RNA degradation assay they find that two mRNAs belonging to the P53 pathway, Aen and Bbc3, are specifically degraded by Exosc10 in cortical NSCs. They observe that Eosc10 cKO leads to decreased numebr of viable neurons without affecting apical and basal progenitors and that death of neurons can be rescued by the inhibitor of p53-dependent transcriptional activation of apoptosis Pifithrin-α. These findings robustly support a role of Exosc10 in supprting cell viability in early cortical development. However, apart the crucial effect on cell viability of Exosc10 inactivation, a direct role of it in cell patterning and differentiation doesn not emerge form the study, limiting its impact on cortical development.

Comments for the author

Telencephalon-specific Exosc10 cKO in FoxG1-Cre mice show no telencephalon, in line with constitutive rather than cell-type specific anti-apototic effect. However, Exm1-Cre directed cKO does not affect progenitors, suggesting instead a differential Exosc10 activity in progenitors vs neurons. I think this is a crucial point that should be further investigated to highlight direct Exosc10 role in developmental mechanisms. I think that a careful analysis of Exosc10 expression in cKO_Exm1-Cre mice should first be performed to rule out the possibility that residual Exosc10 activity is present in progenitors. Then, Aen detection should be carried out to assay wheter progenitor cell survival is due to unefficient Exosc10-directed Aen degradation. If this is the case, addressing the mechanisms of such diversity between progenitors and neurons would be extremely interesting in terms of developmental mechanisms. Finally, a study of the phenotype when inhibiting the P53 pathway in cKO_Exm1-Cre mice could highlight interesting developmental targets of Exosc10. In this line, the Authors might for instances extend their analysis of Pifithrin-α-treated cKO_Exm1-Cre mice and compare the proportions of different types of pyramidal neurons between them and wt mice.

Reviewer 2

Advance summary and potential significance to field

The paper by Ulmke and others details a new new factor which controls apoptotic cell death in the developing telencephalon. The authors focused on the post-transcriptional machinery and RNA decay to reveal its role. Based on a previous finding that RNA exosomes play a major role on gene expression in the developing brain, they concentrated on the ring-like structured exosome complex. Exosc10, which is the catalytic subunit of the complex, showed strong expression in telencephalic VZ of the developing mouse brain. Furthermore, they used Exosc10 cKO and further analyzed its function, revealing that lack of Exosc10 increases apoptotic cell death and reduces telencephalic volume. Using mutant mice with a milder phenotype, they showed which cortical layer or subpopulations are most affected by variety of cortical markers. Moreover, using RNA-seq and RIP-seq, they revealed candidate downstream genes of Exosc10 activated apoptotic molecular pathway. From these results, they identified Aen and Bbc3, which function as a p53 dependent apoptotic signaling pathway [insert word here], as candidate molecules. Therefore, they tried to KO these factors to see whether they can rescue the phenotype of Exosc10 cKO. However, KD Aen, by shRNA construct, was not sufficient to rescue the cKO. Therefore, they concluded that Aen and Bbc3 work as a partial Exosc10 triggered p53 mediated apoptotic signaling pathway. However, it is not the only pathway (which was also shown by the rescue experiment of p53 inhibitor applied mutant mouse). Overall, finding the role of Exosc10 on apoptotic break in developing telencephalon and revealing its target gene is novel, although the importance of the post-transcriptional cell death pathway is not well described. For instance, What kind of occasion switches on this pathway? What triggers the pathway?, Do they specifically choose Exosc10 dependent pathway to control specific cell death? Without addressing these fundamental characteristics, it is too premature and incomplete for publication in Development.

© 2021. Published by The Company of Biologists under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/).

Comments for the author

Specific comments.
(1) As already described above, it lacks why they have to have Exosc10 for p53 dependent cell death break. One of the well-known cell death triggering factors in developing brain is known to be oxidative stress. The authors need to test whether oxidative stress suppresses Exosc10 and induces cell death.
(2) The authors only focused on embryonic stages, however, there are quite a lot of cell death occurring in the postnatal period. Without any data that shows no Exosc10 expression in postnatal cortex, they should look at the function of Exosc10 in postnatal brain as well. Cell death in the postnatal brain is controlled by different factors, such as neuronal activity, and they need to look at the relationship with these as well.
(3) The authors attempted a rescue experiment of cKO by administrating p53 inhibitor from quite early embryonic stage (Fig. 7). They successfully reduced number of cells which showed Casp3 expression, however, the size of the brain looks similar to cKO. They have stated in the discussion about this and speculated there are unknown perturbations which contributed for the reduction of the size. For this kind of analysis, they need to show quantitative analysis of the brain size and volume. Also, they need to show this rescue experiment down regulated Aen and Ddc3.
(4) Although Exosc10 is expressed in entire VZ, cKO showed regional Casp3 increase in dorsal telencephalon only (Fig 4A). Are there any explanations why there is no Casp3 activation in lateral telencephalon?

Minor comments.
Fig 2. Are these sections really from the same region indicate in white box? They look different and are not aligned.
Typo in page 10, line 229. “rescue”

<u>Reviewer 3</u>

Advance summary and potential significance to field

In this manuscript, Ulmke et al investigate the role of the exosome complex in cerebral cortical development using conditional knock out of Exosc 10, a nuclear component of the exosome. Roles of this protein have been studied in cancer and other organs but little is known about the functions in the brain. There is still relatively little knowledge of the functions of RNA regulation in the control of neuronal numbers and types to which this study contributes.

Comments for the author

The work is of merit and the results are interesting. The design of the experiments is in general good, it has the added value of in vivo, and performance and presentation are correct. The analysis of mice with the dorso-telencephalic knock out of Exosc10 allows demonstrating the role of Exosc10 in progenitor and neuronal survival. The results obtained with the RNA seq and RIP-seq analyses showing that the mean-life of P53 targets are controlled by Exosc10 protein are very nice and uncover the likely mechanisms.
Before publication, there are some issues that need to be revised.

Major:
1. Rescue experiments are formally correct and intend to nail down Exosc10 downstream pathway. However, the results turned out not to be informative. I disagree with how the authors develop this part. In the first set of experiments in which they knockdown Aen in cells Exosc10 KO cells, they observe that there are no changes in apoptosis. Here the authors should be more cautious in their conclusions. They do not validate the efficiency of the shRNA that they employed. Therefore, there is always the possibility of an inefficiency knockdown of Aen. Besides, under the assumption of good shRNA efficiency, the results show that knocking down Aen is not sufficient to counteract the loss of Exosc10 per se. In both cases, I would consider that the actual conclusion is that this experiment cannot be used to support or discard the regulation of Aen by Exosc10. The author conclusions “These data suggest that exosc10 suppresses cell death in the developing cortex

© 2021. Published by The Company of Biologists under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/).

by degrading a set of apoptosis-related transcripts, rather than Aen alone" is secondary. I recommend that the authors revise this part either by removing this experiment or by revising the text. I would not oppose to include it, as negative results are interesting, but the conclusions should be carefully revised.
2. In the second part of these rescue experiments shown in Fig 7, there is an important lack of controls. Data showing the number of apoptotic cells in controls treated with P53 inhibitors should be quantified and shown. This is important to the contribution of p53 to normal development, to evaluate the degree of rescue and the alternative that compensatory mechanisms decreasing apoptosis in general, give an apparent rescue.
3. In the result section, ln 164-165 please describe at the beginning of RIP-seq experiments the rationale of this experiment (it is now in a subsequent paragraph ln 174-175). State that these are performed with anti-Exosc10 antibodies. In the Materials and Methods section please describe better this experiment and do not just refer to previous publications.
4. In Fig2G please specify if quantifications are performed in the rostral or caudal cortex.
5. In Fig3 D please show quantifications of rostral and caudal levels separately. It seems that the quantifications do not reflect the alterations in Sox2 and Pax6 cells observed at the caudal levels in the picture.
6. In figure 6 please reformat panels (A-B) in order to be able to increase the readability of the genes affected by Exosc10. Letter size is too small and green is difficult to read. For panel D please the image does not allow to see the quality of the staining. Please add or substitute with a low magnification image that includes dorsal and ventral telencephalon in order to verify the specificity of the staining and selective down-regulation in KO cells. quantify in both regions.
7. I would consider that a nice addition to the manuscript, would be to include if possible, stainings of Exosc10 at embryonic stages in the VZ and cortical plate and in precursors and neuronal subpopulations. This would help to define and reinforce the specific roles of Exosc10 in development. it will help to clarify if this RNA regulation is necessary in precursors, postmitotic cells or both.

First revision

Author response to reviewers' comments

Responses to Reviewers

We are thankful to the reviewers for their thoughtful criticism on several issues, which we took into very careful consideration in preparing the revised version of the manuscript (MS). As pointed out in our detailed answers, we have addressed the comments and concerns of all three reviewers. To facilitate the revision process, the new text added in the revised MS is marked in red.

Response to questions common to the Reviewers

Reviewer 1 (Point 3): I think that a careful analysis of Exosc10 expression in cKO_Exm1-Cre mice should first be performed to rule out the possibility that residual Exosc10 activity is present in progenitors. Then, Aen detection should be carried out to assay whether progenitor cell survival is due to inefficient Exosc10-directed Aen degradation. If this is the case, addressing the mechanisms of such diversity between progenitors and neurons would be extremely interesting in terms of developmental mechanisms.

Reviewer 3 (Point 7): I would consider that a nice addition to the manuscript, would be to include if possible, staining of Exosc10 at embryonic stages in the VZ and cortical plate and in precursors and neuronal subpopulations. This would help to define and reinforce the specific roles of Exosc10 in development. it will help to clarify if this RNA regulation is necessary in precursors, postmitotic cells or both.

Answer: We thank the reviewer for pointing out an important issue in our manuscript. In fact, we were interested in examining the expression of Exosc10 in cortical cell subtypes. To assess the

© 2021. Published by The Company of Biologists under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/).

Exosc10 expression in the developing mouse cortex, we have tested many commercially available Exosc10 antibodies (e.g. Cat. 16731-1-AP, Protein tech; Cat. ab50558, Abcam; Cat. LS-C31532, LifeSpan BioSciences; Cat. bs- 13120R, Bioss Inc, Cat. HPA028484, Sigma) in different conditions. Unfortunately, we cannot provide antibody-based staining for Exosc10, because all tested antibodies did not work with our protocols.

As alternative approach, we examined the expression of Exosc10 and other exosome genes in the published scRNA-seq dataset of the mouse developing cortex (Telley et al. 2016). Consistence with ISH data (Fig. S1B-J), outcome from the analysis revealed that exosome genes, including Exosc10, express widely with their highest expression found in apical progenitors (APs). The new data is described in Fig. S1K- S, page 5.

Reviewer 1 (Point 4): Finally, a study of the phenotype when inhibiting the P53 pathway in cKO_Exm1-Cre mice could highlight interesting developmental targets of Exosc10. In this line, the Authors might for instances extend their analysis of Pifithrin- α-treated cKO_Exm1-Cre mice and compare the proportions of different types of pyramidal neurons between them and wt mice.

Reviewer 2 (Point 3): The authors attempted a rescue experiment of cKO by administrating p53 inhibitor from quite early embryonic stage (Fig. 7). They successfully reduced number of cells which showed Casp3 expression, however, the size of the brain looks similar to cKO. They have stated in the discussion about this and speculated there are unknown perturbations which contributed for the reduction of the size. For this kind of analysis, they need to show quantitative analysis of the brain size and volume. Also, they need to show this rescue experiment down regulated Aen and Bbc3.

Answer: We thank the Reviewers for their very important suggestions. While both Aen and Bbc3 are P53 pathway-related genes, only Bbc3 is a direct target of P53 as the promoter region of Bbc3 contains P53-binding sites and can be directly activated by P53 (Han et al. 2001). We therefore compared the Bbc3 expression of cortices from control and cKO embryos, which were treated with either Veh or P53 inhibitor. As expected, treatment with PFTα decreases the expression of Bbc3, which is upregulated in cKO cortex (Fig. S5C).

To address whether Exosc10 regulates cortical development partly via suppression of P53 signaling, pregnant mice between 9.5 and 15.5 days post coitum (d.p.c.) were intraperitoneally injected daily with PFTα solution. Due to the perinatal lethality of PFTα-treated animals, the brain samples were collected at E18.5 for phenotype analysis (Fig. 8A). The expression of Satb2, which mark majority of projection neurons in all cortical layers and areas (Alcamo et al. 2008; Britanova et al. 2008), was examined in control, and cKO with and without PFTα treatment (Fig. 8B/C). The treatment of P53 inhibitor did not influence the size of WT (control) cortex, as revealed by Satb2 expression (Fig. 8D/E). Remarkably, compared to Veh-treated cKO embryo, PFTα-treated embryo has significantly bigger cortical size (Fig. 8D/E). Concurrently, PFTα administration in cKO mutants resulted in an increase in the number of Satb2$^+$ neurons (Fig. 8F/G). The findings indicated that treatment with the P53 inhibitor partly rescued the aberrant cortical morphology in mutants. The new findings for the rescue experiment are presented in Fig. S5C, Fig. 8, page 11.

Responses to Reviewer 1

Point 1: Apart from the crucial effect on cell viability of Exosc10 inactivation, a direct role of it in cell patterning and differentiation does not emerge from the study, limiting its impact on cortical development.

Answer: As a catalytic subunit of RNA degradosome complex, we believed that Exosc10 plays important roles in various developmental processes of the brain. As captured in the title, our manuscript focused on essential role Exosc10 in cell viability. According, pharmacological inhibition of P53 signaling rescued the described defects in cell viability in the Exosc10cKO mutants (Fig. 7A–C), hence making us posit that the Exosc complex negatively regulates P53 signaling during early cortical development.

It is worth noting, however, that the inhibition of P53 signaling was not able to restore the normal

© 2021. Published by The Company of Biologists under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/).

thickness of cortical layers in Exosc10cKO mutants (Fig. 7, Fig. 8). Therefore, the observed drastic reduction in the size of the cortical plate cannot be singularly ascribed to the increased apoptosis but other unreported perturbationsmay contribute to the observed defective neurogenesis. In agreement with that assertion, our RNA-seq and RIP-seq data suggest that Exosc10 might directly suppress expression of many neuronal differentiation-associated genes. Possible defects in neurogenesis and neuronal differentiation in the cortex-specific Exosc10cKO mutants will be in focus in a separate study. The above information is described in discussion in page 17of our revised manuscript.

In our ongoing study, we aim at understanding how Exosc10 controls other aspects of forebrain development apart from cell viability. Indeed, we found that Exosc10 has important roles in cortical neurogenesis and in dorsal/ventral patterning as loss of Exosc10 in cortex-specific cKO_Emx1-Cre leads to increased neuronal differentiation (below Fig. A/B) and increased expression of ventral-fated genes (below Fig. C) in developing cortex. The underlying mechanisms by Exosc10 in the above developmental events are currently being investigated and will be presented in a separate manuscript.

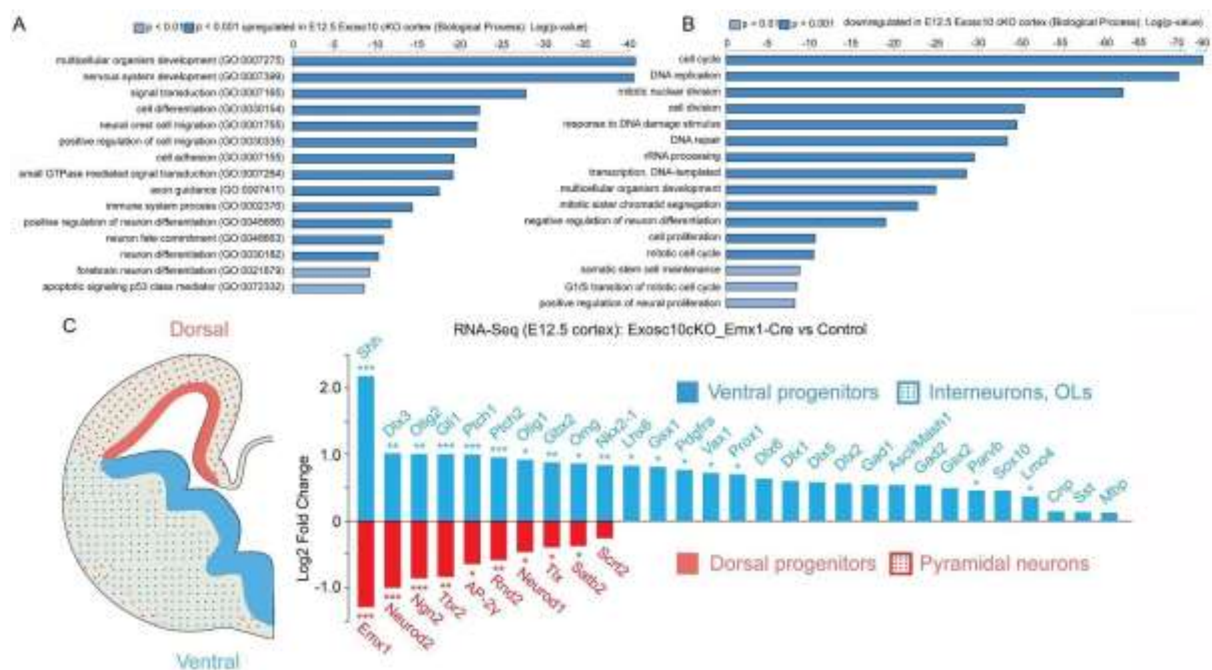

***Figure. Potential roles of Exosc10 in neuronal differentiation and forebrain patterning*.**

(A, B) Gene ontology analysis of genes upregulated, including many neuronal differentiation pathways
(A) and downregulated, including cell proliferation pathways (B) in RNA-Seq analysis for Exosc10cKO_Emx1-Cre cortex at E12.5. (C) Loss of Exosc10 in cortex-specific cKO_Emx1-Cre resulted in decreased expression of dorsal-fated genes and increased expression of ventral-fated genes

Point 2: Telencephalon-specific Exosc10cKO in FoxG1-Cre mice show no telencephalon, in line with constitutive rather than cell-type specific anti-apoptotic effect. However, Exm1-Cre directed cKO does not affect progenitors, suggesting instead a differential Exosc10 activity in progenitors vs neurons. I think this is a crucial point that should be further investigated to highlight direct Exosc10 role in developmental mechanisms.

Answer: Following the suggestions of the reviewers, we further investigated a possible mechanism underlying telencephalic structure is not formed in Exosc10cKO_FoxG1- Cre mutants at E17.5. We examined the formation of telencephalon at early stages, immunohistochemistry (IHC) analysis was performed with antibodies against Sox2, Pax6, HucD, and NeuN in forebrain tissue of control and cKO_*FoxG1*-Cre embryos between E10.5 and E12.5 (Fig. S2). The expression of these markers were

© 2021. Published by The Company of Biologists under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/).

found in telencephalon (Tel), diencephalon (Di) and mesencephalon (Mes) of control embryo, whereas their expression is seen only in Di and Mes structures in cKO_*FoxG1*-Cre mutants. The finding suggests that the deletion of Exosc10 at the onset of telencephalon formation in cKO_*FoxG1*-Cre embryo, this structure is not specified. The new data is presented in Fig. S2 and Pages 5-6.

Responses to Reviewer 2

Point 1: As already described above, it lacks why they have to have Exosc10 for p53 dependent cell death break. One of the well-known cell death triggering factors in developing brain is known to be oxidative stress. The authors need to test whether oxidative stress suppresses Exosc10 and induces cell death.

Answer: Our findings highlighted the presence of Exosc10 is essential for viability of cortical cells. To search for the apoptosis-related target transcripts that are suppressed by Exosc10, we performed RNA-seq and RIP-seq (Fig. 5). Based on the GO analysis for our upregulated genes, we identified two apoptosis-related pathways, which are enriched: (1) signal transduction by p53 class mediator (GO:0072331; enrichment Score: 1.89; pValue= 0.002) and (2) regulation of apoptotic signaling pathway (GO:2001233; enrichment Score: 1.57; pValue= 0.03). Among the transcripts belonging to the P53 and apoptosis pathways, Aen and Bbc3 were directly bound and degraded by Exosc10 (Fig. 6). Our data suggests that usually Aen and Bbc3 are highly transcripted in cortical cells, triggering apoptosis. The main function of the RNA exosome complex is to modulate gene expression during development by controlling the richness of RNAs. To support viability of cortical cells and to ensure the physiological expression level of Aen and Bbc3, Exosc10 degrades the excess level of these transcripts in developing cortex.

As suggested by the Reviewer, in addition to apoptotic P53 signaling pathway, we examined the oxidative stress signaling, one of the well-known cell death triggering pathway in developing brain (Green 1998; Ikonomidou 2009). Notably, expression of genes encoding for main components of this pathway (e.g. BAX, BH3, Cytochrome C) was unchanged in our RNA-seq analysis and the related pathway itself was not found in corresponding GO study (Table S1, S2). Thus, our finding suggests that Exosc10 inhibits apoptosis mainly by suppressing the activity of apoptotic P53 signaling pathway. We added discussion of the apoptosis-related oxidative stress signaling in pages 16-17.

Point 2 : The authors only focused on embryonic stages, however, there are quite a lot of cell death occurring in the postnatal period. Without any data that shows no Exosc10 expression in postnatal cortex, they should look at the function of Exosc10 in postnatal brain as well. Cell death in the postnatal brain is controlled by different factors, such as neuronal activity, and they need to look at the relationship with these as well.

Answer: Following the suggestion of the Reviewer, we compared the apoptosis between control and cKO_Emx1-Cre cortex at postnatal stages. As shown in Fig. S3, there was no difference in the number of Casp3+ apoptotic cells between control and Exosc10cKO cortices at P6, indicating that Exosc10 expression is required for cell viability only during embryonic cortical development. The new data is presented in Fig. S3 and pages 7-8.

Point 4: Although Exosc10 is expressed in entire VZ, cKO showed regional Casp3 increase in dorsal telencephalon only (Fig 4A). Are there any explanations why there is no Casp3 activation in lateral telencephalon?

Answer: Emx1 is a well-known cortical patterning factor, which displays a gradient expression pattern from rostromedial cortex^{high} to caudolateral cortex^{low} (Bishop et al. 2002; Stocker and O'Leary 2016). The Emx1-Cre line was generated by knocking-in Cre cDNA into Emx1 locus (Gorski et al. 2002). Thus, the Cre activity in Emx1-Cre transgenic animals mimics the endogenous expression of Emx1 with the recombinase activity found highest in medial cortex and low in lateral cortex (Gorski et al. 2002). This could account for the loss of Exosc10 in Exosc10cKO_Emx1-Cre (current study) or of BAF complex in BAFcKO_Emx1-Cre (Narayanan et al. 2015; Nguyen et al. 2018) leading to predominance of the attendant apoptosis in rostromedial cortex. We added this plausible explanation in page 7.

© 2021. Published by The Company of Biologists under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/).

Minor comment 1: Fig 2. Are these sections really from the same region indicate in white box? They look different and are not aligned.

Answer: All close-up images were taken from dorsal area of rostral cortex as indicated in white box. As our IHC analyses with different antibodies was performed in different sections, there is a bit of variation in cortical thickness between sections in control cortex. Because the structure of the cortex in the mutants is very small and malformed, the variation is even more obvious in cKO cortex.

Minor comment 1: Typo in page 10, line 229. “rescue”

Answer: The typo has been corrected.

Responses to Reviewer 3

Point 1: Rescue experiments are formally correct and intend to nail down Exosc10 downstream pathway. However, the results turned out not to be informative. I disagree with how the authors develop this part. In the first set of experiments in which they knockdown Aen in cells Exosc10 KO cells, they observe that there are no changes in apoptosis. Here the authors should be more cautious in their conclusions. They do not validate the efficiency of the shRNA that they employed. Therefore, there is always the possibility of an inefficiency knockdown of Aen. Besides, under the assumption of good shRNA efficiency, the results show that knocking down Aen is not sufficient to counteract the loss of Exosc10 per se. In both cases, I would consider that the actual conclusion is that this experiment cannot be used to support or discard the regulation of Aen by Exosc10. The author conclusions “These data suggest that exosc10 suppresses cell death in the developing cortex by degrading a set of apoptosis-related transcripts, rather than Aen alone” is secondary. I recommend that the authors revise this part either by removing this experiment or by revising the text. I would not oppose to include it, as negative results are interesting, but the conclusions should be carefully revised.

Answer: We completely agree with the Reviewer’s opinion that the results of Aen knockdown in Exosc10 KO cells in the rescue experiment were not informative. Following the suggestion of the reviewer, we removed the figure and corresponding text in the revised manuscript.

Point 2: The second part of these rescue experiments shown in Fig 7, there is an important lack of controls. Data showing the number of apoptotic cells in controls treated with P53 inhibitors should be quantified and shown. This is important to the contribution of p53 to normal development, to evaluate the degree of rescue and the alternative that compensatory mechanisms decreasing apoptosis in general, give an apparent rescue.

Answer: As suggested by the Reviewer, we quantified the number of apoptotic cells in control treated with the P53 inhibitor. Because of apoptotic cells are very rare in WT cortex, our quantification indicated that there is no difference in number of Casp3+ cells between Veh- and PFTα- treated control cortices. The image for PFTα-treated control cortex and the corresponding quantification were presented in Fig. 7B/C.

Point 3: the result section, ln 164-165 please describe at the beginning of RIP-seq experiments the rationale of this experiment (it is now in a subsequent paragraph ln 174-175). State that these are performed with anti-Exosc10 antibodies. In the Materials and Methods section please describe better this experiment and do not just refer to previous publications.

Answer: Paragraphs between lines 162-176 (or lines 181-196 in revised manuscript) have been amended to avoid repetition. The RIP-seq experiment is described in detail in pages 18-19.

Point 4: Fig2G please specify if quantifications are performed in the rostral or caudal cortex.

Answer: In Fig. 2G, the quantifications were performed in the dorsal area at rostral level as shown in white box. We specified the cortical area for quantifications in the Fig. 2G legend.

© 2021. Published by The Company of Biologists under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/).

Point 5: Fig3 D please show quantifications of rostral and caudal levels separately. It seems that the quantifications do not reflect the alterations in Sox2 and Pax6 cells observed at the caudal levels in the picture.

Answer: We thank the Reviewer for bringing this important issue to our attention, which we have clarified in the revised manuscript. In our double IHC analysis, we found that expression of Pax6/HuCD (Fig. 3A) and Sox2/NeuN (Fig. 3B) outlined clearly the Pax6+, Sox2+ NSC population in VZ and HuCD+, NeuN+ neurons in CP in the control cortex. Strikingly, in cKO cortex, many Pax6+, Sox2+ cells were also immunoreactive with HuCD and NeuN (Fig. 3A, white arrows). The detail analysis of mis-regulated expression of NSC and neuronal genes in the Exosc10-ablated cortex is currently ongoing. As suggested by the Reviewer, we quantify number of Pax6+/HuCD-, Pax6-/HuCD-, Pax6+/HuCD+, Sox2+/NeuN-, Sox2-/NeuN+, and Sox2+/NeuN+ in rostral and caudal levels, separately (Fig. 3D/E).

Point 6: figure 6 please reformat panels (A-B) in order to be able to increase the readability of the genes affected by Exosc10. Letter size is too small and green is difficult to read. For panel D please the image does not allow to see the quality of the staining. Please add or substitute with a low magnification image that includes dorsal and ventral telencephalon in order to verify the specificity of the staining and selective down-regulation in KO cells and quantify in both regions.

Answer: To increase the readability of the gene name, letter size in Fig.6 (panels A-C) was increased and the green is changed to black

We admit that Aen antibody is not the best antibody. In addition, Aen is a cytoplasm protein, which make it difficult to obtain a high-quality immunostaining. As suggested by the Reviewer, we added low magnification images that indicate dorsal and ventral telencephalic regions, and quantifications in both regions have been included as presented in Fig. 6D/E.

References

Alcamo EA, Chirivella L, Dautzenberg M, Dobreva G, Farinas I, Grosschedl R, McConnell SK. 2008. Satb2 regulates callosal projection neuron identity in the developing cerebral cortex. *Neuron* **57**: 364-377.
Bishop KM, Rubenstein JL, O'Leary DD. 2002. Distinct actions of Emx1, Emx2, and Pax6 in regulating the specification of areas in the developing neocortex. *J Neurosci* **22**: 7627-7638.
Britanova O, de Juan Romero C, Cheung A, Kwan KY, Schwark M, Gyorgy A, Vogel T, Akopov S, Mitkovski M, Agoston D et al. 2008. Satb2 is a postmitotic determinant for upper- layer neuron specification in the neocortex. *Neuron* **57**: 378-392.
Gorski JA, Talley T, Qiu M, Puelles L, Rubenstein JL, Jones KR. 2002. Cortical excitatory neurons and glia, but not GABAergic neurons, are produced in the Emx1- expressing lineage. *J Neurosci* **22**: 6309-6314.
Green DR. 1998. Apoptotic pathways: the roads to ruin. *Cell* **94**: 695-698.
Han J, Flemington C, Houghton AB, Gu Z, Zambetti GP, Lutz RJ, Zhu L, Chittenden T. 2001. Expression of bbc3, a pro-apoptotic BH3-only gene, is regulated by diverse cell death and survival signals. *Proc Natl Acad Sci U S A* **98**: 11318-11323.
Ikonomidou C. 2009. Triggers of apoptosis in the immature brain. *Brain & development* **31**: 488-492.
Narayanan R, Pirouz M, Kerimoglu C, Pham L, Wagener RJ, Kiszka KA, Rosenbusch J, Seong RH, Kessel M, Fischer A et al. 2015. Loss of BAF (mSWI/SNF) Complexes Causes Global Transcriptional and Chromatin State Changes in Forebrain Development. *Cell Rep* **13**: 1842-1854.
Nguyen H, Kerimoglu C, Pirouz M, Pham L, Kiszka KA, Sokpor G, Sakib MS, Rosenbusch J, Teichmann U, Seong RH et al. 2018. Epigenetic Regulation by BAF Complexes Limits Neural Stem Cell Proliferation by Suppressing Wnt Signaling in Late Embryonic Development. *Stem Cell Rep* **10**: 1734-1750.
Stocker AM, O'Leary DD. 2016. Emx1 Is Required for Neocortical Area Patterning. *PLoS One* **11**: e0149900.
Telley L, Govindan S, Prados J, Stevant I, Nef S, Dermitzakis E, Dayer A, Jabaudon D. 2016. Sequential transcriptional waves direct the differentiation of newborn neurons in the mouse neocortex. *Science* **351**: 1443-1446.

© 2021. Published by The Company of Biologists under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/).

Second decision letter

MS ID#: DEVELOP/2020/188276

MS TITLE: Post-transcription regulation by the exosome complex is required for cell survival and forebrain development by repressing P53 signaling

AUTHORS: Pauline Antonie Ulmke, Yuanbin Xie, Godwin Sokpor, Linh Pham, Orr Shomroni, Joachim Rosenbusch, Tea Berulava, Andre Fischer, Uttiya Basu, Huu Phuc Nguyen, Jochen Staiger, and Tran Tuoc

I have now received all the referees reports on the above manuscript, and have reached a decision. The referees' comments are appended below, or you can access them online: please go to BenchPress and click on the 'Manuscripts with Decisions' queue in the Author Area.

The overall evaluation is positive and we would like to publish a revised manuscript in Development, provided that the referees' comments can be satisfactorily addressed. Please attend to all of the reviewers' comments in your revised manuscript and detail them in your point-by-point response. If you do not agree with any of their criticisms or suggestions explain clearly why this is so.

We are aware that you may currently be unable to access the lab to undertake experimental revisions. If it would be helpful, we encourage you to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating where you are able to address concerns raised (either experimentally or by changes to the text) and where you will not be able to do so within the normal timeframe of a revision. We will then provide further guidance. Please also note that we are happy to extend revision timeframes as necessary.

Reviewer 1

Advance summary and potential significance to field

The authors performed a series of new experimets that fulfill my requests answering the major points that I raised. I think that this supplemental work significantly improved the manuscript.

Comments for the author

none

Reviewer 3

Advance summary and potential significance to field

In this manuscript, Ulmke et al investigate the role of Exosc 10, a key protein of the exosome complex, in cerebral cortical development. They analyze a telencephalic knock out of Exosc 10. The work helps to understand the functions of RNA regulation in the control of neuronal numbers and neuronal types.

Comments for the author

I thank the authors for their work in providing a revised version of the manuscript. I consider that the revisions have significantly improved the manuscript. Still, there are some confusing aspects pertaining to the main mechanistic claims of the manuscript that need to be addressed before publication.

Major issues:

© 2021. Published by The Company of Biologists under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/).

1-Data showing how the expression of Bcb3 is down-regulated after pharmacological inhibition of p53 is a very important addition to this version. This part of Fig S5 should be included in the main Figures. It supports the model proposed by the authors.
They have not analyzed the expression of Aen as they argue that it is not a direct target. I do consider that this argument is incorrect. In the manuscript they established Aen as a target of the Exoc10, and then claimed that this finding is important because its part of the p53 mediated survival of cortical cells. (Line 204 "many genes involved in P53 apoptosis signaling (e.g., Ccng1, 205 Sesn2, Pmaip1, Bbc3 and Aen)". Thereafter, they focus on AEN and Bcb3.
AEN RNA stability shows a clear dependency on Exoc10 expression. By contrast, the kinetics of Bcb3 stability in Exoc10 mutants show only differences at late time points.
I consider that it is important to analyze Aen expression under the pharmacological p53 inhibition. Directly or indirectly, it is expected that Aen is likely downregulated by pharmacological inhibition of p53. In case it is not, data is still relevant it should be discussed, as it would indicate parallel converging pathways regulating cortical survival. The authors should include the analysis of Aen expression upon p53 inhibition in cortical cells. It calls my attention that Aen expression is very low according to RNAseq, which questions the biological meaning of these changes. Perhaps the authors can also discuss this.

2-I consider that the results obtained with the Foxg1 conditional deletion should be moved to supplementary data. Without rescue experiments, they do not inform about the mechanisms involved and are only suggestive and do not really help to make the point of the manuscript.

3-The data related to the expression pattern of Exo10c on the other hand can be better presented in the main section. In the main results, perhaps it is better to restrict this part to the description of the expression of Exosc10. Here, perhaps they should comment on why they do not detect the expression of all the other components of the exosome in the same cells, if, as suggested by their scheme of the protein, all the components are required for exonuclease activity.

4-Please improve Figure 6 panel D. Do not show only Aen green staining (include DAPI and/or outline cortical plate, vz, etc..). Also, when describing this panel in the result section, do not refer to it as showing Aen and Bcb3 immunohistochemistry.

Second revision

Author response to reviewers' comments

Responses to Reviewer 1

Comment: The authors performed a series of new experiments that fulfill my requests, answering the major points that I raised. I think that this supplemental work significantly improved the manuscript.

Answer: We thank the reviewer for the positive comment, and for the constructive suggestions throughout the review process.

Responses to Reviewer 3

Point 1: Data showing how the expression of Bcb3 is down-regulated after pharmacological inhibition of p53 is a very important addition to this version. This part of Fig S5 should be included in the main Figures. It supports the model proposed by the authors. They have not analyzed the expression of Aen as they argue that it is not a direct target. I do consider that this argument is incorrect. In the manuscript they established Aen as a target of the Exoc10, and then claimed that this finding is important because its part of the p53 mediated survival of cortical cells. (Line 204 "many genes involved in P53 apoptosis signaling (e.g., Ccng1, 205 Sesn2, Pmaip1, Bbc3 and Aen)". Thereafter, they focus on AEN and Bcb3. AEN RNA stability shows a clear dependency on Exoc10 expression. By contrast, the kinetics of Bcb3 stability in Exoc10 mutants show only differences at

© 2021. Published by The Company of Biologists under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/).

late time points. I consider that it is important to analyze Aen expression under the pharmacological p53 inhibition. Directly or indirectly, it is expected that Aen is likely downregulated by pharmacological inhibition of p53. In case it is not, data is still relevant it should be discussed, as it would indicate parallel converging pathways regulating cortical survival. The authors should include the analysis of Aen expression upon p53 inhibition in cortical cells. It calls my attention that Aen expression is very low according to RNAseq, which questions the biological meaning of these changes. Perhaps the authors can also discuss this.

Answer: We thank the reviewer for this very important suggestion to analyze the expression of Aen upon p53 inhibition in cortical cells, which would strengthen our conclusions for distinct Exoc10 and p53-dependent pathways regulating cell viability in the developing cortex. In addition to Bbc3, we compared the expression of Aen from control and cKO cortices, which were treated with either Veh or P53 inhibitor. In contrast to the Bbc3 expression (Fig. 7E), PFTα treatment did not significantly rescue the aberrant upregulation of Aen upon the loss of Exosc10 in the developing cortex (Fig. 7D). This is in line with the evidence that Bbc3 (but not Aen) is a direct target of P53 since the promoter region of Bbc3 contains P53-binding sites and can be directly activated by P53 (Han et al. 2001).
As suggested by the reviewer, we present data for the rescue experiment in main Fig. 7D/E and in pages: 11, 16, 17 (lines: 241-249, 386-392).

Point 2: I consider that the results obtained with the Foxg1 conditional deletion should be moved to supplementary data. Without rescue experiments, they do not inform about the mechanisms involved and are only suggestive and do not really help to make the point of the manuscript.

Answer: Following the suggestions of the reviewer, we moved the results obtained with the Foxg1 conditional deletion (Fig. 1A, in previous submission) to the supplementary figure 2.

Point 3: The data related to the expression pattern of Exosc10 on the other hand can be better presented in the main section. In the main results, perhaps it is better to restrict this part to the description of the expression of Exosc10. Here, perhaps they should comment on why they do not detect the expression of all the other components of the exosome in the same cells, if, as suggested by their scheme of the protein, all the components are required for exonuclease activity.

Answer: We thank the reviewer for the very important suggestions, which would improve our data presentation and open up future investigations into the function of exosome complex. In our revised manuscript, we have presented data related to the expression pattern of Exosc10 in the main figure 1A-B. In addition, we included in the Discussion section (page 15, lines 344-359) the suggested comment on possible consequence of different expression pattern of exosome subunits in the developing cortex as following: “The ring-like structured exosome complex contains eleven evolutionary- conserved subunits, including nine structural subunits (Exosc1-9) and two catalytic subunits (Exosc10, and Dis3) (Januszyk and Lima 2014; Kilchert et al. 2016). The expression pattern analysis (Fig. S1B-J) revealed that many exosome subunits (e.g. Exosc1, Exosc2, Exosc3, Exosc5, Exosc9, Exosc10) are widely expressed in the developing mouse cortex. Remarkably, expression of some subunits is found restrictedly in the VZ (Exosc8) or SVZ (Exosc7). This raises question whether all the components are required for the RNA exonuclease activity of exosome complex. Even though our understanding of the functions of exosome complex and its subunits in development has improved, several key questions remain enigmatic. For instance, is the composition of the exosome complex restricted to eleven subunits? Also, what is the contribution of individual subunits in formation and action of exosome complex? Whether there is possible existence of lineage-restricted subunits leads to dynamic combinatorial-assembly of exosome complexes, producing their biological specificity, is yet to determined. Effort to resolve these and other questions would stimulate continuous interest in this area of research.

Point 4: Please improve Figure 6 panel D. Do not show only Aen green staining (include DAPI and/or outline cortical plate, vz, etc..). Also, when describing this panel in the result section, do not refer to it as showing Aen and Bcb3 immunohistochemistry.
Answer: As suggested, we included images with DAPI and with labeling of CP, VZ in Fig. 6D. In addition, we revised the sentence (on pages 9 and 10, lines 213-217) as following: “Their upregulated expression in the Exosc10cKO cortex was first revealed by RNA-seq (Fig. 6B), then

© 2021. Published by The Company of Biologists under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/).

confirmed by qPCR (for Bbc3, Aen Atr, Fig. 6C) and IHC analyses (for Aen, Fig. 6D, E), making them strong candidates for mediating regulation of apoptosis by Exosc10”.

References

Alcamo EA, Chirivella L, Dautzenberg M, Dobreva G, Farinas I, Grosschedl R, McConnell SK. 2008. Satb2 regulates callosal projection neuron identity in the developing cerebral cortex. *Neuron* **57**: 364-377.
Bishop KM, Rubenstein JL, O'Leary DD. 2002. Distinct actions of Emx1, Emx2, and Pax6 in regulating the specification of areas in the developing neocortex. *J Neurosci* **22**: 7627-7638.
Britanova O, de Juan Romero C, Cheung A, Kwan KY, Schwark M, Gyorgy A, Vogel T, Akopov S, Mitkovski M, Agoston D et al. 2008. Satb2 is a postmitotic determinant for upper- layer neuron specification in the neocortex. *Neuron* **57**: 378-392.
Gorski JA, Talley T, Qiu M, Puelles L, Rubenstein JL, Jones KR. 2002. Cortical excitatory neurons and glia, but not GABAergic neurons, are produced in the Emx1- expressing lineage. *J Neurosci* **22**: 6309-6314.
Green DR. 1998. Apoptotic pathways: the roads to ruin. *Cell* **94**: 695-698.
Han J, Flemington C, Houghton AB, Gu Z, Zambetti GP, Lutz RJ, Zhu L, Chittenden T. 2001. Expression of bbc3, a pro-apoptotic BH3-only gene, is regulated by diverse cell death and survival signals. *Proc Natl Acad Sci U S A* **98**: 11318-11323.
Ikonomidou C. 2009. Triggers of apoptosis in the immature brain. *Brain & development* **31**: 488-492.
Narayanan R, Pirouz M, Kerimoglu C, Pham L, Wagener RJ, Kiszka KA, Rosenbusch J, Seong RH, Kessel M, Fischer A et al. 2015. Loss of BAF (mSWI/SNF) Complexes Causes Global Transcriptional and Chromatin State Changes in Forebrain Development. *Cell Rep* **13**: 1842-1854.
Nguyen H, Kerimoglu C, Pirouz M, Pham L, Kiszka KA, Sokpor G, Sakib MS, Rosenbusch J, Teichmann U, Seong RH et al. 2018. Epigenetic Regulation by BAF Complexes Limits Neural Stem Cell Proliferation by Suppressing Wnt Signaling in Late Embryonic Development. *Stem Cell Rep* **10**: 1734-1750.
Stocker AM, O'Leary DD. 2016. Emx1 Is Required for Neocortical Area Patterning. *PLoS One* **11**: e0149900.
Telley L, Govindan S, Prados J, Stevant I, Nef S, Dermitzakis E, Dayer A, Jabaudon D. 2016. Sequential transcriptional waves direct the differentiation of newborn neurons in the mouse neocortex. *Science* **351**: 1443-1446.

Third decision letter

MS ID#: DEVELOP/2020/188276

MS TITLE: Post-transcription regulation by the exosome complex is required for cell survival and forebrain development by repressing P53 signaling

AUTHORS: Pauline Antonie Ulmke, Yuanbin Xie, Godwin Sokpor, Linh Pham, Orr Shomroni, Joachim Rosenbusch, Tea Berulava, Andre Fischer, Uttiya Basu, Huu Phuc Nguyen, Jochen Staiger, and Tran Tuoc
ARTICLE TYPE: Research Article

I am happy to tell you that your manuscript has been accepted for publication in Development, pending our standard ethics checks.

© 2021. Published by The Company of Biologists under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/).