An indispensable role for dynamin-related protein 1 in beige and brown adipogenesis

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Original submission

First decision letter

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MS TITLE: An indispensable role for dynamin-related protein 1 (DRP1) in adipogenesis

AUTHORS: Raja Gopal Reddy Mooli, Dhanunjay Mukhi, Zhonghe Chen, Nia Buckner, and Sadeesh Kumar Ramakrishnan
ARTICLE TYPE: Research Article

We have now reached a decision on the above manuscript.

As you will see, the reviewers raise a number of substantial criticisms that prevent me from accepting the paper at this stage. They suggest, however, that a revised version might prove acceptable, if you can address their concerns. If you think that you can deal satisfactorily with the criticisms on revision, I would be pleased to see a revised manuscript. We would then return it to the reviewers.

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.

Please 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. Please attend to all of the reviewers' comments. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

Reviewer 1

Advance summary and potential significance to field

The manuscript submitted by Raja Gopal Reddy Mooli et al. describes that mitochondrial fission protein dynamin-related protein 1 (DRP1) is important for adipocyte differentiation. The inhibition of DRP1 using Mdivi-1 mitigates adipocyte differentiation and differentiation-associated mitochondrial biogenesis with impairing induction of the early phase of adipogenic transcriptional program.

Comments for the author

The manuscript is original. It improves the understanding of the importance of DRP1 during the early phase of adipocytes differentiation. Although the data and the discussion are clearly presented, some questions need to be addressed to strengthen their manuscript as follows:

Major concerns:
1) In the Figure1, the authors showed that DRP1 is highly expressed in brown and beige adipocytes, also higher in differentiated adipocytes than preadipocytes. However, in Figure2 and the following figures, DRP1 was inhibited by mdivi to add culture media from day0 to day2 or day2 to day4 if I understand correctly. Impaired adipogenesis by inhibiting DRP1 was observed when treatment was done during early periods of differentiation, not maintenance periods. Does this mean even a small amount of DRP1 plays an important role in preadipocyte? Or, the more early-stage when the author did not observe (e.g. 1, 2, 6, 12, 24 hr after differentiation), DRP1 may transiently be induced, then inhibition of induced DRP1 in the early stage results in the phenotype authors observed?
2) Genetical loss of function experiment is required to exclude the off-target effect of the pharmacological inhibition.

Minor points:
1) Figure 1B: better to have a border between “vehicle” or “CL”, although I guess n=5 vs n=4.
2) Among the 2 bands of immunoblot for DRP1, which is considered to be functionally important? Is that due to the splice variant?
3) In Figure 1F, DRP1 and Actin protein in BAT samples seem to shift down, does that occur naturally?
4) It might be easier to understand if the information of mitochondrial size (MitoTracker etc.) and mitochondrial number (qPCR) after mdivi treatment are available.
5) It seems there are faint scale bars in the adipocytes pictures. It is better to have a scale each picture.
6) It is better to clearly mention in the figure legend on which day after differentiation all the assays were done. I guess most of the data are done on Day 6 after differentiation.

Although this is a difficult time to concentrate research, I believe the manuscript submitted by Raja Gopal Reddy Mooli et al. would strengthen after address the concerns/questions, could be acceptable.

Reviewer 2

Advance summary and potential significance to field

This study shows that DRP1 is essential for the induction of the early phase of the adipogenic transcriptional program and differentiation-associated mitochondrial biogenesis of brown adipocytes.

Comments for the author

In this paper Mooli et al. have shown that mitochondrial fission protein dynamin-related protein 1 (DRP1) is highly expressed in brown adipose tissue and its expression increases during brown adipocyte differentiation. The authors have shown that DRP1 is essential for the induction of the

early phase of the adipogenic transcriptional program and differentiation-associated mitochondrial biogenesis. Whereas, after the induction of adipogenesis DRP1 is dispensable for adipogenesis and adipogenesis-associated mitochondrial biogenesis. Overall, the study is interesting and systematically performed to show the role of DRP1 in the adipogenesis of brown adipocytes. However, there is confusion between brown and beige 'adipogenesis'. Therefore, before acceptance, the following comments need to be addressed.

Comments:

1. In the abstract authors claim that DRP1 plays a role in brown and beige adipogenesis, whereas there is no data on beige adipogenesis. It is clear that DRP1 increases during beiging, but is it involved in the transformation of white to beige adipocytes, or is it involved in beige adipogenesis? Though, the data is clear on the role of DRP1 on the adipogenesis of brown adipocytes.
2. The title needs to be more specific because the study shows that DRP1 plays a role in brown adipogenesis.
3. In the discussion, authors say that "This is consistent with the previous study, where knockdown of DRP1 in the mature adipocytes did not affect the adipocyte differentiation (Pisani et al., 2018)". A paragraph later they say, "To our knowledge, the role of DRP1 in adipocyte differentiation has never been explored". This indicates an inconsistency in the statements which need to be corrected.
4. The result of Fig. 2B is not clearly defined - "Moreover, the mRNA levels of other brown fat-specific genes such as Ucp1, Prdm16, Cidea, and Dio2 in the adipocytes differentiated in the presence of Mdivi-1 (Figure 2B)".
5. In Fig. 3c, the PPARg plot is either missing or not visible.

First revision

Author response to reviewers' comments

We thank the Editor and Reviewer for allowing us to revise and resubmit the manuscript. We have marked the major changes in the manuscript in blue and the response to the comments below in blue.

Reviewer 1

The manuscript is original. It improves the understanding of the importance of DRP1 during the early phase of adipocytes differentiation. Although the data and the discussion are clearly presented, some questions need to be addressed to strengthen their manuscript as follows:

We thank the Reviewer for appreciating our effort in manuscript originality and its importance during the brown adipogenesis.

Major concerns:

1) In the Figure1, the authors showed that DRP1 is highly expressed in brown and beige adipocytes, also higher in differentiated adipocytes than preadipocytes. However, in Figure2 and the following figures, DRP1 was inhibited by mdivi to add culture media from day0 to day2, or day2 to day4 if I understand correctly. Impaired adipogenesis by inhibiting DRP1 was observed when treatment was done during early periods of differentiation, not maintenance
periods. Does this mean even a small amount of DRP1 plays an important role in preadipocyte? Or, the more early-stage when the author did not observe (e.g. 1, 2, 6, 12, 24 hr
after differentiation), DRP1 may transiently be induced, then inhibition of induced DRP1 in the early stage results in the phenotype authors observed?

We thank the reviewer for the question. We assessed the expression of DRP1 during early stages of adipocyte differentiation as the Reviewer suggested and found that the induction of DRP1 is taking place as early as 6 hourr and further significantly increased at 12 hour (Figure 4C). Thus, the data

from strongly suggests that early induction of DRP1 play a critical role in the initiation of adipogenesis.

2) Genetical loss of function experiment is required to exclude the off-target effect of the pharmacological inhibition.
We agree with the Reviewer's concern. We performed the siRNA-mediated knockdown of Drp1 in SVFs cells and show that knockdown of DRP1 phenocopies the effect of Mdivi-1 such as down regulation of genes involved in the thermogenesis, mitochondrial content and mitochondrial dynamics (Figure 2G), lipid accumulation (Figure 3D & E). This further validates our finding that DRP1 is essential for brown adipocyte differentiation.

Minor points:
1) Figure 1B: better to have a border between "vehicle" or "CL", although I guess n=5 vs n=4.
We have labeled in the figure.

2) Among the 2 bands of immunoblot for DRP1, which is considered to be functionally important? Is that due to the splice variant?
Yes, the Reviewer is correct. DRP1 has been shown to have splice variants (PMID: 26578513). Therefore, we believe that the two bands observed in the DRP1 immunoblot could be due to their splice variant. Although, each isoform is shown to have different GTPase activities, their functions significance are not known.

3) In Figure 1F, DRP1 and Actin protein in BAT samples seem to shift down, does that occur naturally?
As per our observation, we saw a slight shift in the blots when we run BAT and WAT samples in the same gel. The BAT and WAT lysates were prepared at the same time and the samples were loaded and Western blots were resolved at the same time in one single gel. Therefore, these shifts should not be due to technical reasons. We believe that the abundant expression of DRP1 and low expression of actin in the BAT samples as a potential reason that causes the slight shift in the position of proteins.

4) It might be easier to understand if the information of mitochondrial size (MitoTracker etc.) and mitochondrial number (qPCR) after mdivi treatment are available.
We agree with Reviewer and for that we incubated the differentiated the adipocytes in the presence or absence of Mdivi-1 with Mitotracker Green FM and found that the fluorescence intensity was higher in differentiated alone compared to the Mdivi-1 treated. However, we were unable the measure the exact mitochondrial size with the available microscope, since, university advanced microscope core facility was closed due to COVID-19 pandemic. Regarding the mitochondrial number, our data from (Figure 2C and D) strongly supports the notion that Drp1 is essential for the mitochondrial biogenesis (thereby content) during the adipocyte differentiation. Therefore, we strongly believe that treatment of adipocytes with Mdivi-1 results in reduction in the mitochondrial number.

5) It seems there are faint scale bars in the adipocytes pictures. It is better to have a scale each picture.
We apologize for the quality of the scale bars. We have included scale bars of appropriate size for all the images.

6) It is better to clearly mention in the figure legend on which day after differentiation all the assays were done. I guess most of the data are done on Day 6 after differentiation.
We have mentioned in all the figure legends

Although this is a difficult time to concentrate research, I believe the manuscript submitted by Raja Gopal Reddy Mooli et al. would strengthen after address the concerns/questions, could be acceptable.

Once again, we thank the reviewer for the positive feedback.

Reviewer 2

In this paper Mooli et al. have shown that mitochondrial fission protein dynamin-related protein 1 (DRP1) is highly expressed in brown adipose tissue and its expression increases during brown adipocyte differentiation. The authors have shown that DRP1 is essential for the induction of the early phase of the adipogenic transcriptional program and differentiation-associated mitochondrial biogenesis. Whereas, after the induction of adipogenesis DRP1 is dispensable for adipogenesis and adipogenesis-associated mitochondrial biogenesis. Overall, the study is interesting and systematically performed to show the role of DRP1 in the adipogenesis of brown adipocytes. However, there is confusion between brown and beige 'adipogenesis'. Therefore, before acceptance, the following comments need to be addressed.

We thank the reviewer for appreciating our work

1) In the abstract authors claim that DRP1 plays a role in brown and beige adipogenesis, whereas there is no data on beige adipogenesis. It is clear that DRP1 increases during beiging, but is it involved in the transformation of white to beige adipocytes, or is it involved in beige adipogenesis? Though, the data is clear on the role of DRP1 on the adipogenesis of brown adipocytes.

We thank the Reviewer for the question. In our study, we have not addressed the role of DRP1 in transformation of white adipocytes to brown adipocytes. In this study, we differentiated primary and transformed SVF cells from inguinal adipose tissue into beige adipocytes and demonstrate that DRP1 is essential for beige adipocyte differentiation. We now added data to show that Mdivi-1 also inhibits differentiation of brown adipocytes using SVF cells isolated from brown adipose tissue (Supplement Figure 1 B-D). We have made it clear in the revised version. Although not assessed in this study, we recently demonstrated that white to beige adipocyte transition during calorie restriction is associated with an increase in the expression of DRP1 in the white adipose tissue (PMID: 32275973). Further studies using adipose tissue-specific DRP1 KO mice is required to determine if DRP1 has differential role in regulating beige and brown adipocyte induction and maintenance.

2) The title needs to be more specific because the study shows that DRP1 plays a role in brown adipogenesis.

We have changed the title to "An indispensable role of DRP1 in beige and brown adipogenesis".

3) In the discussion, authors say that "This is consistent with the previous study, where knockdown of DRP1 in the mature adipocytes did not affect the adipocyte differentiation (Pisani et al., 2018)". A paragraph later they say, "To our knowledge, the role of DRP1 in adipocyte differentiation has never been explored". This indicates an inconsistency in the statements which need to be corrected.

We apologize for the inconsistency in the statement. The cited literature determined the role of DRP1 in rosiglitazone-mediated conversion of mature white adipocytes to beige. Here, we determined the role of DRP1 in beige adipocyte differentiation from the adipocyte precursor cells. We have addressed this in the revised discussion.

4) The result of Fig. 2B is not clearly defined - "Moreover, the mRNA levels of other brown fat-specific genes such as Ucp1, Prdm16, Cidea, and Dio2 in the adipocytes differentiated in the presence of Mdivi-1 (Figure 2B)".

We have addressed this.

5) In Fig. 3c, the PPARg plot is either missing or not visible.

We have corrected the plot.

Second decision letter

MS ID#: JOCES/2020/247593

MS TITLE: An indispensable role for dynamin-related protein 1 (DRP1) in beige and brown adipogenesis

AUTHORS: Raja Gopal Reddy Mooli, Dhanunjay Mukhi, Zhonghe Chen, Nia Buckner, and Sadeesh Kumar Ramakrishnan
ARTICLE TYPE: Research Article

I am happy to tell you that your manuscript has been accepted for publication in Journal of Cell Science, pending standard ethics checks.

Reviewer 1

Advance summary and potential significance to field

This study improves the understanding of the importance of DRP1 during the early phase of adipocytes differentiation.

Comments for the author

In the revision, additional works: especially the expression of DRP1 during the early stages of adipocyte differentiation, and genetic loss of function experiment made this paper strengthen more. I think the authors worked very well during this pandemic time. Almost all my concerns are clear. Not related to this manuscript it might be interesting to test/identify function of each among the double bands of DRP1 for future study. I think this version of the manuscript is great enough to publish in the Journal of Cell Science.

Reviewer 2

Advance summary and potential significance to field

The authors have shown that DRP1 is essential for the induction of the early phase of the adipogenic transcriptional program and differentiation-associated mitochondrial biogenesis.

Comments for the author

My previous comments have been addressed in the revised manuscript.