The SRCAP chromatin remodeling complex promotes oxidative metabolism during prenatal heart development

Mingjie Xu, Jie Yao, Yingchao Shi, Huijuan Yi, Wukui Zhao, Xinhua Lin and Zhongzhou Yang
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MS ID#: DEVELOP/2020/199026

MS TITLE: The SRCAP chromatin remodeling complex promotes oxidative metabolism during prenatal heart development

AUTHORS: Mingjie Xu, Jie Yao, Yingchao Shi, Huijuan Yi, Wukui Zhao, Xinhua Lin, and Zhongzhou Yang

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.

We are aware that you may be experiencing disruption to the normal running of your lab that make experimental revisions challenging. 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 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.

Reviewer 1

Advance summary and potential significance to field

During embryogenesis, cardiomyocytes make a metabolic shift from anaerobic glycolysis towards oxidative metabolism. This work by Xu et al explores the regulators that promote this mitochondrial shift. They specifically focus on the SRCAP chromatin remodeling complex member, Znhit1 and find that cardiomyocyte specific loss of Znhit1 results in cardiac developmental abnormalities leading to heart failure by E18.5 and peri natal lethality. In these models, they demonstrate mitochondrial stress and show that some genes relevant to mitochondrial metabolism are misregulated upon loss of Znhit1. While the work is very promising and focuses on the very interesting process of cardiomyocyte maturation, it does not convey any specific mechanistic insight.

Comments for the author

Major comments:

1. The study begins with a characterization of the specific developmental window during which mitochondrial maturation and the switch to oxidative metabolism is likely to occur. However, it is unclear how SRCAP and Znhit1 emerge as candidate regulators from this work.
a. How does expression of Znhit1 and other SRCAP members change between E10.5-E18.5?
b. What fraction of the assayed RNA in the bulk RNA Seq data is mitochondrial? If the authors subsetted just the MitoCarta 2.0 genes, what patterns of expression would emerge and which genes specifically exhibit dynamic regulation during this critical window?
2. In Fig 4, the authors curiously find that expression of some hand picked respiratory chain subunits is changed in mutant cells at the post transcriptional level without providing any insight on how a chromatin regulator affect protein stability. Eg: are there any candidate factors that might regulate these genes as a secondary effect of loss of Znhit1?
3. In Fig 5, 95 candidate mitochondrial genes are identified as downregulated in two models of cardiomyocyte-specific loss of Znhit1. How many of these 95 are also directly affected in the CUT&Tag assay?

Minor comments:
1. In Fig 2G and H, more details about the method should be included. At what age were the cells harvested for protein quantification and were whole hearts or cardiomyocytes used for this?
2. Text should be edited for minor grammatical errors

Reviewer 2

Advance summary and potential significance to field

The manuscript entitled “The SRCAP chromatin remodeling complex promotes oxidative metabolism during prenatal heart development” represents a well-organized and careful study of the Snf2-related CREBBP Activator Protein (SRCAP) complex during late cardiac development. The authors describe a critical temporal window during which the developing heart shifts from glycolytic metabolism to oxidative phosphorylation and show that the SRCAP chromatin remodeling complex is critical for this adjustment. Using two Cre-dependent genetic models with the deletion of Znhit1, a regulatory subunit of the SRCAP complex, in combination with transcriptomic and H2A.Z DNA-binding analyses, the authors demonstrate that the SRCAP complex is critical in regulating the transcription of key genes required for mitochondrial maturation and the TCA cycle.

Overall, this study provides a thorough and important advancement in the understanding of how chromatin remodeling plays a role in heart development and maturation. This work could provide an important reference for future studies. While the experiments described in this manuscript are interesting and generally well-described, this submission would be strengthened if the following details were included:

Comments for the author

The manuscript entitled “The SRCAP chromatin remodeling complex promotes oxidative metabolism during prenatal heart development” represents a well-organized and careful study of the Snf2-related CREBBP Activator Protein (SRCAP) complex during late cardiac development. The authors describe a critical temporal window during which the developing heart shifts from glycolytic metabolism to oxidative phosphorylation and show that the SRCAP chromatin remodeling complex is critical for this adjustment. Using two Cre-dependent genetic models with the deletion of Znhit1, a regulatory subunit of the SRCAP complex, in combination with transcriptomic and H2A.Z DNA-binding analyses, the authors demonstrate that the SRCAP complex is critical in regulating the transcription of key genes required for mitochondrial maturation and the TCA cycle.

Overall, this study provides a thorough and important advancement in the understanding of how chromatin remodeling plays a role in heart development and maturation. This work could provide an important reference for future studies. While the experiments described in this manuscript are interesting and generally well-described, this submission would be strengthened if the following details were included:

1. The authors mention that the Znhit1-deficient heart results in perinatal lethality. Therefore, it would be helpful to provide the observed frequency of this event for the cTnT-Cre-mediated KO animals at E16.5 and P1, to better understand the extent of the penetrance of lethality.
2. The authors mention and show multiple consequences such as mitochondrial metabolic gene regulation that are consistent with Mef2c-AHF-Cre-mediated KO of Znhit1. Does the Mef2c-AHF- Cre-mediated KO display a similar timeline of lethality? It would also be helpful to describe whether proliferation or non-compaction defects in the right ventricle are observed in this model.
3. In figure 5, the authors performed Gene Ontology enrichment analysis and present a Venn- diagram showing down-regulated genes in the KO. Because up-regulated genes in the KO constitute approximately 40% of the transcriptome changes, an analysis of the up-regulated genes would provide a more complete picture of the global transcriptome changes.
4. In figure 6 panel C, the authors highlighted five loci of metabolic genes. While some of the highlighted representations of promoter binding have an obvious diminished peak in the KO (e.g., Coq3 and Echs1), the other plots do not contain clear peaks, which may be due to the considerable number of background reads. The authors should display the peak regions that have been unbiasedly defined using programs such as MACS2 or HOMMER, along with the displayed chromatin landscape.
5. What is the overall percentage of the promoter-associated H2A.Z peak that is decreased in KO versus control? Is there an overlap between the genes with a decreased H2A.Z peak and those with decreased expression in the KO? It would be helpful to describe this point in the text.
6. Are there any transcription factor motifs enriched in the differentially-bound H2A.Z peaks? This would be important to help explain potential SRCAP-dependent transcription factor regulation for metabolic genes.

Minor comments:

1. Scale for the y-axis of the binding track is needed in Figure 6 panel C. Panel B of this figure is too small.
2. Individual points are provided in some graphs (e.g., Figure 3 D and E), but not others (e.g., Figure 2 B, D, F, and H; Figure 6 D and F). These should be included uniformly throughout the manuscript.

3. How is ventricular wall thickness defined in Figure 3 panel D? Is this the measurement for the compact-zone or a combination of the compact-zone and trabeculae of the myocardium?

First revision

Author response to reviewers' comments

Reviewer #1:
During embryogenesis, cardiomyocytes make a metabolic shift from anaerobic glycolysis towards oxidative metabolism. This work by Xu et al explores the regulators that promote this mitochondrial shift. They specifically focus on the SRCAP chromatin remodeling complex member, Znhit1 and find that cardiomyocyte specific loss of Znhit1 results in cardiac developmental abnormalities leading to heart failure by E18.5 and peri natal lethality. In these models, they demonstrate mitochondrial stress and show that some genes relevant to mitochondrial metabolism are misregulated upon loss of Znhit1. While the work is very promising and focuses on the very interesting process of cardiomyocyte maturation, it does not convey any specific mechanistic insight.

Response: We thank you for careful and critical evaluation of our study. Your suggestions and comments are constructive, which helps improve the quality of this work.

Comments:
1. The study begins with a characterization of the specific developmental window during which mitochondrial maturation and the switch to oxidative metabolism is likely to occur. However, it is unclear how SRCAP and Znhit1 emerge as candidate regulators from this work.

Response: This is a good question. One of our research focuses is to study the cell fate determination of the second heart field (SHF) progenitors during heart development. Originally, we wanted to know whether SRCAP and Znhit1 regulated this process and deleted *Znhit1* in the SHF progenitors. However, we failed to define an important function of SRCAP and Znhit1 in this aspect. Instead, we disclosed the new function of this complex in cardiomyocyte oxidative metabolism.
The SRCAP complex is one of the three complexes in the INO80 chromatin remodeling family, and Dr. Ashby J. Morrison and colleagues at Stanford have made comprehensive investigations of the yeast INO80 chromatin remodeling complex in metabolism, which has provided good reference for our study.
Therefore, we moved from studying SRCAP/Znhit1 in cardiac progenitor development to cardiomyocyte oxidative metabolic regulation.

3. How does expression of Znhit1 and other SRCAP members change between E10.5- E18.5?
Response: To address this question, we performed Western blot analysis to study the temporal expression patterns of Znhit1, YL-1 and H2A.Z. The results demonstrated significantly increased levels of these proteins at E11.5 and E12.5, which was in consistence with the metabolic shift window defined by us (see result below). These data were integrated in the Fig. S2 of the revised manuscript.

We have removed unpublished data provided for the reviewers in confidence.

4. What fraction of the assayed RNA in the bulk RNA Seq data is mitochondrial? If the authors subsetted just the MitoCarta 2.0 genes, what patterns of expression would emerge and which genes specifically exhibit dynamic regulation during this critical window?

Response: Nearly 5% (4.46%) of the assayed mRNAs was mitochondrial and they were predominantly included in Cluster 2 and 3. In addition, we carried out a thorough analysis of these mitochondrial genes and categorized them into three clusters. Cluster A were genes responsible for assembly of mitochondrial inner membrane and complexes, and associated with tRNA metabolism. The expression level of this cluster was declining steadily, suggesting that the process of mitochondrial maturation was towards completion. Cluster B and C enriched a large amount of oxidative metabolic genes whose

expression patterns indicated that in the mid-term of gestation, part of them were first activated to regulate metabolic shift and the others were subsequently induced to maintain oxidative metabolism. This data was added in Fig. S1 (below).

We have removed unpublished data provided for the reviewers in confidence.

2. In Fig 4, the authors curiously find that expression of some hand picked respiratory chain subunits is changed in mutant cells at the post transcriptional level without providing any insight on how a chromatin regulator affect protein stability. E.g.: are there any candidate factors that might regulate these genes as a secondary effect of loss of Znhit1?
Response: This is a good question. We observed aberrant mitochondrial cristae in the cardiomyocytes of *Znhit1*-deletion mice (Fig. 4A and 4B). It was previously reported that mitochondrial cristae remodeling and damage would disrupt the respiratory super- complexes (*Cogliati S.* et al. 2013), and we therefore speculate that the reduced protein levels of the respiratory chain subunits might be a consequence of defective mitochondrial cristae.
On the other hand, Cardiolipin is the signature phospholipid of mitochondria and is abundantly enriched in the inner mitochondrial membrane for maintenance of the respiratory super-complexes (*Paradies G.* et al. 2014; *Kasahara T.* et al. 2020). Cardiolipin is synthesized by cardiolipin synthase (encoded by *Crls1*). We performed a detailed study of *Crls1* in our CUT & Tag assay data, and found with surprise, that *Crls1* expression could be directly regulated by the SRCAP complex. Afterwards, we examined the expression levels of *Crls1* and detected a significant reduction upon deletion of *Znhit1* (see results below). Based on these results, it is plausible to propose that reduction of *Crls1* expression might impair the stability of the respiratory complexes as a second effect to *Znhit1* loss of function.

These points were added in the Fig. S9 and in the Discussion.

We have removed unpublished data provided for the reviewers in confidence.

References:
1. Cogliati, S., Frezza, C., Soriano, M. E., Varanita, T., Quintana-Cabrera, R., Corrado, M., ... & Scorrano, L. (2013). Mitochondrial cristae shape determines respiratory chain supercomplexes assembly and respiratory efficiency. *Cell*, *155*(1), 160-171.
2. Paradies, G., Paradies, V., Ruggiero, F. M., & Petrosillo, G. (2014). Cardiolipin and mitochondrial function in
health and disease. *Antioxidants & redox signaling*, *20*(12), 1925-1953.
3. Kasahara, T., Kubota‐Sakashita, M., Nagatsuka, Y., Hirabayashi, Y., Hanasaka, T., Tohyama, K., & Kato, T. (2020). Cardiolipin is essential for early embryonic viability and mitochondrial integrity of neurons in mammals. *The FASEB Journal*, *34*(1), 1465-1480.

3.In Fig 5, 95 candidate mitochondrial genes are identified as downregulated in two models of cardiomyocyte-specific loss of Znhit1. How many of these 95 are also directly affected in the CUT&Tag assay?
Response: The CUT&Tag assay identified that 39 genes among the 95 candidate mitochondrial genes were directly affected. This number was integrated into the revised text.

Minor comments:
1. In Fig 2G and H, more details about the method should be included. At what age were the cells harvested for protein quantification and were whole hearts or cardiomyocytes used for this?
Response: The specific information was added in the figure legends as 'Heart ventricular tissues from E13.5 embryos'.

2. Text should be edited for minor grammatical errors
Response: This was done.

In the end, we thank you once more for evaluating our work and for your help with this

manuscript.

Reviewer #2:
The manuscript entitled "The SRCAP chromatin remodeling complex promotes oxidative metabolism during prenatal heart development" represents a well-organized and careful study of the Snf2-related CREBBP Activator Protein (SRCAP) complex during late cardiac development. The authors describe a critical temporal window during which the developing heart shifts from glycolytic metabolism to oxidative phosphorylation and show that the SRCAP chromatin remodeling complex is critical for this adjustment. Using two Cre-dependent genetic models with the deletion of Znhit1, a regulatory subunit of the SRCAP complex, in combination with transcriptomic and H2A.Z DNA-binding analyses, the authors demonstrate that the SRCAP complex is critical in regulating the transcription of key genes required for mitochondrial maturation and the TCA cycle.
Overall, this study provides a thorough and important advancement in the understanding of how chromatin remodeling plays a role in heart development and maturation. This work could provide an important reference for future studies. While the experiments described in this manuscript are interesting and generally well- described, this submission would be strengthened if the following details were included.

Response: We thank this reviewer for investing time and efforts to evaluate of our work, and for appreciation of this study. Your comments and suggestions are constructive and critical, which helps improve the quality of this work.

1. The authors mention that the Znhit1-deficient heart results in perinatal lethality. Therefore, it would be helpful to provide the observed frequency of this event for the cTnT-Cre-mediated KO animals at E16.5 and P1, to better understand the extent of the penetrance of lethality.
Response: All the *Znhit1*-deficient mice (*cTnT-Cre*-mediated KO) survived to E16.5, but started to die from E17.5. Half of the mice were lost before birth and the rest of them could be born but only survived for less than half a day.
This information was integrated into the text.

2. The authors mention and show multiple consequences such as mitochondrial metabolic gene regulation that are consistent with Mef2c-AHF-Cre-mediated KO of Znhit1. Does the Mef2c-AHFCre-mediated KO display a similar timeline of lethality? It would also be helpful to describe whether proliferation or non-compaction defects in the right ventricle are observed in this model.
Response: The *Mef2c-AHF-Cre* mice show Cre activity predominantly in the outflow tract (OFT) and right ventricle (RV), and we did not observed OFT abnormalities in the KO mice. Although these mice demonstrated impaired RV development, the phenotype was much less severe compared to the *cTnT-cre; Znhit1*$^{f/f}$ mice. All of the *Mef2c-AHF- cre; Znhit1*$^{f/f}$ mice could be born, but majority of the mice were lost shortly after birth and around one third of them could survive beyond one week. Cardiomyocyte proliferation was markedly reduced in the RV of KO mice after E12.5 and non-compaction defects was not evident.
These results were integrated in Fig. S5A, 5B and 5F.

3. In figure 5, the authors performed Gene Ontology enrichment analysis and present a Venndiagram showing down-regulated genes in the KO. Because up-regulated genes in the KO constitute approximately 40% of the transcriptome changes, an analysis of the up-regulated genes would provide a more complete picture of the global transcriptome changes.
Response: We performed GO and KEGG enrichment analysis on the 363 genes that were up-regulated in both *Tnnt2*-Cre and *Mef2c-AHF*-Cre mediated *Znhit1* deletion mice. The results were presented in Fig.S7 (below).

We have removed unpublished data provided for the reviewers in confidence.

4. In figure 6 panel C, the authors highlighted five loci of metabolic genes. While some of the highlighted representations of promoter binding have an obvious diminished peak in the KO (e.g., Coq3 and Echs1), the other plots do not contain clear peaks, which may be due to the considerable number of background reads. The authors should display the peak regions that have been unbiasedly

defined using programs such as MACS2 or HOMMER, along with the displayed chromatin landscape.
Response: As you mentioned, the CUT&Tag-seq data was originally processed with the program of MACS2. Distinct from the ChIP-seq assay, CUT&Tag-seq does not need to remove background using IgG, which could bring slightly high noise in some regions of the genome. Nonetheless, direct comparison of the peaks at the same position and on the same background can unbiasedly tell whether there are changes of H2A.Z binding between control and KO groups.

5. What is the overall percentage of the promoter-associated H2A.Z peak that is decreased in KO versus control? Is there an overlap between the genes with a decreased H2A.Z peak and those with decreased expression in the KO? It would be helpful to describe this point in the text.
Response: This is a good question and suggestion. We found that about 76% of the promoter-associated H2A.Z peaks were decreased in the KO compared to control. There are 39 genes where peaks of promoter-binding H2A.Z were decreased substantially among the 95 over-lapping genes. This number was integrated into the revised text.

6. Are there any transcription factor motifs enriched in the differentially-bound H2A.Z peaks? This would be important to help explain potential SRCAP-dependent transcription factor regulation for metabolic genes.
Response: To address this question, a thorough analysis was performed to search for potential transcription factor motifs, and those of the Activator Protein-1 (AP-1) transcription factors, including c-Jun, JunD, ATF2 and ATF7 were enriched in the differentially-bound H2A.Z peaks. AP-1 has been reported to regulate a wide range of cellular processes, including proliferation, differentiation and apoptosis (*Shaulian and Karin,* 2002*)*. Interestingly, it was previously reported that the AP-1 transcription factors were involved in regulating metabolism (*Hasenfuss SC et al*. 2014; *Bozec A. et al*. 2013) Therefore, AP-1 family members could be potential SRCAP-dependent transcription factors in regulating metabolic genes.

References:
1. Hasenfuss, S. C., Bakiri, L., Thomsen, M. K., Williams, E. G., Auwerx, J., & Wagner, E. F. (2014). Regulation of steatohepatitis and PPARγ signaling by distinct AP-1 dimers. *Cell metabolism*, *19*(1), 84-95.
2. Bozec, A., Bakiri, L., Jimenez, M., Rosen, E.D., Catala-Lehnen, P., Schinke, T., Schett, G., Amling, M., and Wagner, E.F. (2013). Osteoblast-specific expression of Fra-2/AP-1 controls adiponectin and osteocalcin expression and affects metabolism. *J Cell Sci 126*, 5432-5440.
3. Shaulian, E., and Karin, M. (2002). AP-1 as a regulator of cell life and death. *Nature Cell Biology 4*, E131- E136.

Minor comments:
1. Scale for the y-axis of the binding track is needed in Figure 6 panel C. Panel B of this figure is too small.
Response: This was corrected.

2. Individual points are provided in some graphs (e.g., Figure 3 D and E), but not others (e.g., Figure 2 B, D, F, and H; Figure 6 D and F). These should be included uniformly throughout the manuscript.
Response: This was done.

3. How is ventricular wall thickness defined in Figure 3 panel D? Is this the measurement for the compact-zone or a combination of the compact-zone and trabeculae of the myocardium?
Response: The ventricular wall thickness refers to that of the compacted zone of the left ventricle, not a combination of the two zones.

In the end, we thank you once more for evaluation of our study, and for the valuable comments and suggestions. Your help with this manuscript is greatly appreciated.

Second decision letter

MS ID#: DEVELOP/2020/199026

MS TITLE: The SRCAP chromatin remodeling complex promotes oxidative metabolism during prenatal heart development

AUTHORS: Mingjie Xu, Jie Yao, Yingchao Shi, Huijuan Yi, Wukui Zhao, Xinhua Lin, and Zhongzhou Yang
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.

Reviewer 1

Advance summary and potential significance to field

This study by Xu et al investigates the mechanism by which a switch in cardiomyocyte metabolism switches from anaerobic glycolosis towards oxidative metabolism. Using transmission electron microscopy, transcriptional analysis and protein validation, they carefully identify the narrow window during embryonic development during which time this metabolic shift takes place. The expression pattern of components of their candidate regulator, SRCAP, were expressed during this same window. Knockdown of the SRCAP core component, Znhit1 ussing Tnnt2 Cre and Mef2c AHF Cre demonstrate morphological impairment of developing heart by E13.5 with perinatal death. Hearts of the mutant mice TEM shows that these hearts have deformed mitochondria. Curiously though, these key mitochondrial genes are reduced at the protein, but not transcriptional level. Transcriptional analysis by RNA Seq demonstrated that several metabolism related genes were downregulated in Znhit1 deficient mice. Since Znhit1 is involved in H2AZ deposition, the authors probe this by CUT&Tag and find that roughly half the mitochondrial gene set analyzed was decreased in mutants. Taken together, this work shows a novel role of a chromatin remodeler, SRCAP in the process of cardiomyocyte metabolic maturation. Although, many questions about the mechanism by which such a regulation would take place remain unanswered, this study uncovers a novel role for a chromatin regulator and presents very interesting findings about the cardiac maturation process.

Comments for the author

The authors have satisfactorily addressed the comments from revision. However the supporting results from the possible role of Cardiolipin should be moved to the results section.

Reviewer 2

Advance summary and potential significance to field

The authors have satisfactorily addressed all my concerns.

Comments for the author

good job