TAF4b transcription networks regulating early oocyte differentiation

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Original submission

First decision letter

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MS TITLE: TAF4b transcription networks regulating early oocyte differentiation

AUTHORS: Megan A Gura, Soňa Relovská, Kimberly M Abt, Kimberly A Seymour, Tong Wu, Haskan Kaya, James MA Turner, Thomas G Fazzio, and Richard Freiman

I have now received all the referees' reports on the above manuscript, and have reached a decision. As you will note, the reviewers have different opinions overall, but agree that the study is of interest to the field and timely. Specifically, Reviewer 3recommends a substantial revision of your manuscript before we can consider publication, which may include some additional analysis or experimentation. If you are able to revise the manuscript along the lines suggested, I will be happy to receive a revised version of the manuscript. Your revised paper will be re-reviewed by one or more of the original referees.

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

The study demonstrates the regulation of early oocyte differentiation by TAF4b transcription networks, connecting several gene regulatory nodes that contribute to the mammalian ovarian reserve. The work would help clarify the best ways to manage certain syndromes of infertility and improve assisted reproduction therapies.

Comments for the author

1. Please double-check entire manuscript and fix any minor typos or errors in grammar, spelling, punctuation, spacing, etc. (for e.g., page 3- para 2, line 3- add space between sentences, page 3- 2nd last line - add space between "we" and "recently", page 17- para 2 - add space between "to" and "dissociate", etc.).
2. Please discuss any limitations of the study and recommend potential future directions to overcome those limitations.
3. How many replicates were each method repeated through, and were all results consistent across all replicates?
4. In methods, please clearly state the sample sizes used for each experiment as appropriate, and also justify the generalizability of the results based on the sample sizes used.
5. Figures - please double-check axes-labels and units if/wherever may be pending.
6. Please double-check mentioning details about all appropriate controls.
7. To increase the cross-domain visibility and citability of this work, potential molecular cross-talks via other direct/indirect interactors of TAF4b, also implicated in mouse oocyte meiosis, could be discussed; for example, 14-3-3 (YWHA) and CDC25B. In this context, please consider citing the following reports suitably:
a) https://doi.org/10.1186/1756-0500-5-57 b) https://doi.org/10.1186/1471-213X-13-10 c) https://doi.org/10.1186/s12861-019-0200-1 d) https://doi.org/10.21467/ias.10.1.52-59

Reviewer 2

Advance summary and potential significance to field

DEVELOP-2021-200074 TAF4b transcription networks regulating early oocyte differentiation Gura A,….Freiman RN

The data presented in this manuscript provide new insights into the gene regulatory mechanisms by which TAF4b impacts early oocyte development and represents a key extension of previous studies from this laboratory. Using state-of-the-art techniques (single cell RNA-seq, gene expression profiling and CUT&RUN gene targeting), the investigators have characterized genes involved in meiosis and DNA repair that are regulated by TAF4b at embryonic age E16.5 and that impact the fertility of female mice and the ovarian reserve.

The manuscript is carefully written and the data are clearly presented. New targets of TAF4b include JunD, Sp1 and Fmr1. Although the functional interactions have not been determined, the associations of TAF4b with Sp1 and Fmr1 are exciting and novel, and in particular provide a link with Fragile X Syndrome and infertility. In addition, genes deregulated in the TAF4b knockout embryos are associated with genes know to be deregulated in Turners Syndrome.

Thus, this manuscript provides a substantial advance for understanding the impact of TAF4b in female and male infertility and embryo development.

Minor comments: the text needs to be proof-read for minor errors where words are linked together.

Comments for the author

No revision is required beyond small issues with words linked together.
Nice study!!

Reviewer 3

Advance summary and potential significance to field

The goal of the manuscript is to analyze the role of Taf4b in developing mouse oocytes. The authors continued in their long-term interest and used several approaches (data from bulk RNA-

Seq, single cell RNA-Seq and CUT&RUN) to compare gene expression of potential Taf4b targets in different models (Taf4b deficient, Turner syndrome). Even though the study showed broad spectrum of tools and datasets, the biological interpretation is unclear and not well explained.

Comments for the author

Manuscript: TAF4b transcription networks regulating early oocyte differentiation describes to role of TAF4b during oocyte formation. I appreciate usage of various tools (bulk and single cell RNA-Seq and CUT&RUN), but connections between different analyses in the result section are not clear to me. Authors performed large scale analysis, but applied only basic bioinformatics to describe DEG, GO terms and compared several datasets. This is usually just a first step leading to identification of genes of interest.
There is no validation of data. In my opinion, qPCR, in situ hybridization or IHC of a few top DEGs would be important to show that datasets are of good quality and that these genes are really affected by treatments/conditions. In addition, I am missing functional analysis of several top candidates in connection with TAF4b LOF phenotype. This would improve biological relevance of discovered genes/pathways or biological processes. Now, the manuscript is rather collection of results from various datasets, without deep description of similarities and differences, which would help understanding of mechanism of Taf4b activity during oocyte differentiation.

Minor comments:
- Introduction part text has many typos (missing gaps after words).

First revision

Author response to reviewers' comments

Response to Reviewers

We would like to thank the reviewers for their valuable input and appreciate the opportunity to utilize their critical feedback to improve upon our initial study. In this revised manuscript, we have re-analyzed the CUT&RUN data to improve the focus of our study on specific pathways and genes in the developing oocyte genome that are regulated by TAF4b, both directly and indirectly. Moreover, we extend the validation of some of these genes at the mRNA level through qRT-PCR and at the protein level by immunofluorescence. With these new data analyses and experimental outcomes in hand, we have focused the study to make more specific conclusions about how TAF4b accesses the oocyte genome and its precise regulation of RNA polymerase II transcription required for establishing the initial primordial follicle pool. In summary, we greatly appreciate the helpful feedback and hope you find the revised manuscript attentive to your previous suggestions.

Specific Responses to Reviewer #1

Reviewer 1 Comments for the Author:

1. Please double-check entire manuscript and fix any minor typos or errors in grammar, spelling, punctuation, spacing, etc. (for e.g., page 3- para 2, line 3- add space between sentences, page 3- 2nd last line - add space between "we" and "recently", page 17- para 2 - add space between "to" and "dissociate", etc.).

We apologize for the spacing and grammar issues detected in our original submission of the manuscript. We have carefully examined the revised version of this manuscript multiple times and have found and corrected these important issues.

2. Please discuss any limitations of the study and recommend potential future directions to overcome those limitations.

We appreciate this comment and agree that this information is very important and should have been in the original discussion. In response, we have added a paragraph addressing the critical limitations and future directions of our study in the revised discussion section.

3. How many replicates were each method repeated through, and were all results consistent across all replicates?

Thank you for the question. We performed bulk RNA-sequencing after three independent embryonic ovary collections at E16.5 and ended up comparing five biological replicates of each genotype. We also completed two independent E16.5 CUT&RUN experiments and these are both better highlighted in revised Figures 2 and 5. Most importantly, by examining the CUT&RUN replicates in more detail as shown revised Figure 5A-C, we are able to conclude that >90% of TAF4b peaks are in promoters of at least 449 genes as shown in the overlap. In addition, the DNA motifs associated with oocyte-specific TAF4b occupancy that resulted from each replicate is now shown in Figure S9A-B which helps demonstrate the reproducibility of our CUT&RUN approach and highlight the importance of these new binding sites for oocyte- and TAF4b-dependent transcription.

4. In methods, please clearly state the sample sizes used for each experiment as appropriate, and also justify the generalizability of the results based on the sample sizes used.

Thank you for the comment and we have added more details about the sample size information (or where to find the sample size information) in both the results and methods sections. We also added sections in the methods section about the generalizability of the results based on the sample sizes used.

5. Figures - please double-check axes-labels and units if/wherever may be pending.

We have completed this.

6. Please double-check mentioning details about all appropriate controls.

We have completed this.

7. To increase the cross-domain visibility and citability of this work, potential molecular cross-talks via other direct/indirect interactors of TAF4b, also implicated in mouse oocyte meiosis, could be discussed; for example, 14-3-3 (YWHA) and CDC25B.

Thank you for this comment and we agree that more could have been done to discuss potential molecular crosstalk via other interactors of TAF4b. Therefore, we have added several sentences in the discussion section about two major motifs that were consistent across our data and the current evidence of their respective proteins' connections to TAF4b: Sp1 and NFY. This is now highlighted as a major finding and also a topic of future studies.

Specific Responses to Reviewer #2

Minor comments: the text needs to be proof-read for minor errors where words are linked together.

We apologize for the spacing issues in the initial submission of this manuscript. We have carefully examined the revised version of this manuscript multiple times and have hopefully found all instances of these previous spacing issues.

Specific Responses to Reviewer #3

Reviewer 3 Comments for the Author:

Manuscript: TAF4b transcription networks regulating early oocyte differentiation describes to role of TAF4b during oocyte formation. I appreciate usage of various tools (bulk and single cell RNA-Seq and CUT&RUN), but connections between different analyses in the result section are not clear to me. Authors performed large scale analysis, but applied only basic bioinformatics to describe

DEG, GO terms and compared several datasets. This is usually just a first step leading to identification of genes of interest. There is no validation of data. In my opinion, qPCR, in situ hybridization or IHC of a few top DEGs would be important to show that datasets are of good quality and that these genes are really affected by treatments/conditions.

These are all excellent points. Our original submission failed to mention our previous work that help validate the protein changes of several candidate genes affected in our current study. These include Nobox and γH2AX, two proteins we have previously published that are decreased and increased, respectively by immunostaining in the TAF4b-deficient embryonic oocytes (Grive et al., 2016). This previous study is now better linked in the revised discussion about validation. We also used real-time PCR and immunostaining to validate that upregulation of Fmr1 in our RNA- seq which we thought was one of the more interesting gene changes when it comes to POI and female fertility. As shown in revised Figure S2 and Figure 6, Fmr1 mRNA and FMRP protein levels are elevated in TAF4b-deficient oocytes in these independent biological samples.

In addition, I am missing functional analysis of several top candidates in connection with TAF4b LOF phenotype. This would improve biological relevance of discovered genes/pathways or biological processes. Now, the manuscript is rather collection of results from various datasets, without deep description of similarities and differences, which would help understanding of mechanism of Taf4b activity during oocyte differentiation.

These are all good suggestions that prompted to analyze our data more in depth which was very productive. We have found that TAF4b may play more of a role as a transcriptional repressor/buffer than as predicted as a co-activator (Fig 6C). We also found that the localization of TAF4b peaks is significantly different between all promoter peaks and peaks that were associated with DEGs (Fig 7C) and that by examining the top motifs relative to the TSS, Sp1 and NFY emerge as two remarkably consistent proteins of interest that may work with TAF4b at an important subset of these promoters (Fig S9E). With the improved analysis on the female CUT&RUN data and its integration with the RNA-seq data we now highlight a critical set of genes that are direct binding targets of TAF4b. While the functional analysis of these identified target genes and transcription factors is beyond the scope of this study, we have also better outlined these future paths in the revised discussion.

Minor comments:

- Introduction part text has many typos (missing gaps after words).

We apologize for the spacing issues in the initial submission of this manuscript. We have carefully examined the revised version of this manuscript multiple times and have hopefully found all instances of this spacing issue.

Second decision letter

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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 2

Advance summary and potential significance to field

This is a timely and interesting study

Comments for the author

The revised manuscript is acceptable.

Reviewer 3

Advance summary and potential significance to field

Authors addressed most of my comments and a new version of manuscript is better. I understand functional validation is another story (time consuming too) and could be published separately. I recommend revised manuscript for publication.

Comments for the author

I do not have more suggestions to authors.