Kinesin motor KIFC1 is required for tubulin acetylation and actin-dependent spindle migration in mouse oocyte meiosis

Meng-Meng Shan, Yuan-Jing Zou, Zhen-Nan Pan, Hao-Lin Zhang, Yi Xu, Jia-Qian Ju and Shao-Chen Sun
DOI: 10.1242/dev.200231

Editor: Haruhiko Koseki

Review timeline
Original submission: 28 September 2021
Editorial decision: 20 December 2021
First revision received: 5 January 2022
Accepted: 18 January 2022

Original submission

First decision letter

MS ID#: DEVELOP/2021/200231

MS TITLE: Kinesin motor KIFC1 is required for tubulin acetylation and actin-dependent spindle migration in mouse oocyte meiosis

AUTHORS: Meng-Meng Shan, Yuan-Jing Zou, Zhen-Nan Pan, Hao-Lin Zhang, Yi Xu, Jia-Qian Ju, and Shao-Chen Sun

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 BenchPressand 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

This study investigated the roles of kinesin motor KIFC1 in mouse oocyte meiosis by depleting KIFC1 expression and rescue approach. The authors showed that KIFC1 is not only essential for proper spindle formation by regulating tubulin acetylation, but also controlled actin-mediated spindle migration and polarity establishment during mouse oocyte maturation. The study provides data revealing novel functions of KIFC1 in meiosis.

Comments for the author

This reviewer has the follwing questions that need the authors to clarify:
The authors need to clarify why they did Nocodazole and Taxol treatment of oocytes.
When microinjection was performed, did the authors put the oocytes in the medium containing milrinone to prevent GVBD? How long were the oocytes cultured after KIFC1-Myc mRNA injection?
Why the authors used 500 μl ovarian lysate to do mass spectrometry (MS) analysis instead og using oocytes?
In the paragraph of KIFC1 is essential for mouse oocyte meiotic maturation (pages 12-13), there are repeated descriptions on the inhibition of polar body extrusion caused by morpholino injection and its rescue by Myc-KIFC1 277 mRNA injection
Page 13, line 288, the authors need to clarify why they investigated spindle formation and chromosome alignment after KIFC1 knockdown.
Page 14, line 305, the authors need to explain why the examined the activity and localization of p-MAPK.
Could the authors reasonably explain why HDAC6 expression decreased and the expression of NAT10 increased in the MO injection group?
Why the authors studied the effects of KIFC1 depletion on ER?
When both KIFC1 activity was inhibited or depleted by MO injection, there were still a proportion of oocytes extruded the polar bodies. What is the reason?
KIFC1 regulated both spindle formation and migration. Is there over-sized polar body?
The figures are in good quality in general. But In Fig 4H and 6H, the loading control actin levels differ in different groups.
The language needs major improvement.

Reviewer 2

Advance summary and potential significance to field

*KIFC1 had a critical role in spindle architecture and chromosome alignment in mouse oocytes independent from the MAPK pathway.
*KIFC1 regulated spindle organization through its effects on tubulin acetylation related proteins during mouse oocytes maturation.
*KIFC1 played a critical role in spindle migration, which in an actin dynamics-dependent manner during oocyte meiotic maturation.
*KIFC1 regulated actin assembly and spindle migration though Fmn2-N-WASP-ARP2/3 complex-based pathway during mouse oocytes maturation.

Comments for the author

The study made a precise aim to prove that K1FC1 was a required factor for mouse oocyte meiosis by finding out the mechanism of K1FC1 related to an actin-dependent pathway. The authors gave an informative background of the asymmetric division in oocyte meiosis and how it related to the function of microtubules and actin filament in spindle formation. The information of microtubule-associated proteins, tubulin acetylation-related proteins (acetyltransferase αTAT, NAT10, HDAC6, Sirt2), and actin filament-related proteins (Arp2/3 complex Formin2, RhoA) were also given to make a background for the research. The most important information was the functions and interaction of K1FC1 in the previous studies were also given that gave rise to the research gap of the role of K1FC1 in mouse oocyte meiosis. The authors evidently found the localization of K1FC1 in

mouse oocyte meiosis which enriched around the spindle. They also proved that K1FC1 was essential for mouse oocyte meiotic maturation due to polar body extrusion defect in the knock-down KFC1 group but not in the GVBD stage.
Furthermore, the study successfully demonstrated that knock-down K1FC1 caused the polar body extrusion failure by affecting the chromosome alignment after altering acetylation tubulin acetylation and spindle migration through Fmn2-N-WASP-ARP2/3 complex-based pathway. On the other hand, rescuing K1FC1 by mRNA injection recover polar body extrusion by fixing chromosome alignment, spindle migration, and their abnormalities-related pathway.
Generally, the study was designed to appropriately answer the aim of finding the function of K1FC1 in mouse oocytes. Adding to the knowledge of the K1FC1 roles on microtubule dynamics in mitosis, the study demonstrated that KIFC1 was important for microtubule acetylation-based spindle stability and played a novel role on actin dynamics and spindle migration through ER-based Fmn2 and ARP2/3 function during mouse oocyte maturation. The research presented a transparent methodology of how the study process. The methods of sample collection treatment groups, immunostaining, and data analysis were well mentioned with reliability and validity. The variable of each experiment was well defined and measured appropriately. In addition, there were enough details that the replication of the study could be replicated.
Although these results are interesting and potentially exciting, some of issues raise concern that aspects of the work do not support the very strong conclusions reached by the authors. Addressing the following issues would substantially improve the manuscript.
*Actually, Kinesin superfamily of proteins (KIFs) have important roles in chromosome separation, microtubule dynamics, spindle formation, cytokinesis, and cell cycle progression. There were reported that Kif5B, Kif1B, Kif4, Kif11 Kif20A, Kif2A are all essential for normal oocyte meiosis in mouse oocytes including GVBD, spindle formation, PB extrusion (Mailhes et al., 2004; Gui and Homer, 2012; Kidane et al., 2013; Zhang et al., 2014; Chen et al., 2016; Kong et al., 2016; Camlin et al., 2017). Importantly, KIFC1 proteins involve in regulating DNA synthesis in S phase, and chromatin maintenance in not only mitosis, but also meiosis such as: KIFC1 is essential for normal spermatogenesis (Hao et al. 2019) or Spindle microtubules-associated protein HSET/KIFC1 labelling along the spindle was reported in mouse oocytes (Yang et al. 2020).
Therefore, the author should discuss the result compared to KIFs related to meiosis in mouse oocyte (For example: KIFC1 was reported in mouse oocytes by Yang et al. 2020 was not cited).
Because the present result 1 and 2 mention about localization of KIFC1 at all stages of oocyte maturation and KIFC1 was essential for mouse oocyte meiotic maturation
*The author reported that involvement of Kif4a in spindle formation and chromosome segregation in mouse oocytes in previous report (2018). The present result 3 show that KIFC1 regulates spindle formation and chromosome alignment in mouse oocytes meiosis. It seems same function between Kif4a and KIFC1 in your research?
*In addition, the author reported Kif18a regulates Sirt2-mediated tubulin acetylation for spindle organization during mouse oocyte meiosis (2018). The present result 4 show that KIFC1 regulated spindle organization through its effects on tubulin acetylation related proteins during mouse oocytes maturation.
What relation of Kif18a and KIFC1 regulated spindle organization through its effects on tubulin acetylation?
*Moreover, the author reported Kif17 regulates tubulin acetylation for spindle organization and drives actin-mediated spindle migration during meiosis (2019).
The present result 5 show that KIFC1 played a critical role in spindle migration, which in an actin dynamics-dependent manner during oocyte meiotic maturation. It seems same function between them.
*Because the authors have some published papers related to KIFs, the authors should make more clearer about regulation of KIFs during oocyte meiotic maturation to reader understand the specific function of KIFs during oocyte meiosis. The author can make graphical abstract about KIFs during oocyte meiotic maturation.
*On the other hand, some points in the article need additional information: the meaning of Nocodazole and Taxol treatment. The author should also make a clarification of using the concentration of AZ82 and K1FC2-MO in this study.
Which factor the author based on to choose this concentration and whether increasing the treatment dose can completely inhibit the polar body extrusion as the results only show the partial effects of K1FC1 knock-down.
*Some minor points in this study that the author should be considering. In figure 3A, there should be staining of K1FC1 to clarify the effects of AZ82 and knock-down treatment. In figure 3D, the

picture of p-MAPK in control-MO looks the same as AZ82 and knock-down treatment, yet the result showed that they were different.
*Please correct Fig number Fig. 1D to 1E and Fig. 1E to 1D.

First revision

Author response to reviewers' comments

Reviewer 1

The authors need to clarify why they did Nocodazole and Taxol treatment of oocytes.

Response: Thank you very much for your positive comments and your remind. To check localization of KIFC1 on microtubules, we used the nocodazole and taxol, which can promote the depolymerization and polymerization of microtubules to confirm, and we added this interpretation in the Results part (L257-260).

When microinjection was performed, did the authors put the oocytes in the medium containing milrinone to prevent GVBD? How long were the oocytes cultured after KIFC1-Myc mRNA injection?

Response: Thank you very much for your remind, yes, for knock down approach we used milrinone to prevent GVBD and we added this information in Methods L168. We also revised the typo mistake for L173, and it should be 4 h.

Why the authors used 500 μl ovarian lysate to do mass spectrometry (MS) analysis instead of using oocytes?

Response: Thank you very much for your question. For MS analysis, large amount of protein lysate was required, however, oocytes contain few protein contents and we could not collect enough samples. We tried using 3,000 oocytes to perform MS, but the results were still negative. Since MS analysis was performed to only predict the functional relationship between proteins, therefore, for oocyte study labs, ovarian lysate was generally used for MS analysis.

In the paragraph of KIFC1 is essential for mouse oocyte meiotic maturation (pages 12-13), there are repeated descriptions on the inhibition of polar body extrusion caused by morpholino injection and its rescue by Myc-KIFC1 277 mRNA injection

Response: Thank you very much for your remind. We deleted the redundant description and re-organized this part.

Page 13, line 288, the authors need to clarify why they investigated spindle formation and chromosome alignment after KIFC1 knockdown.

Response: Thank you very much for your suggestion. We added this interpretation at L294-295.

Page 14, line 305, the authors need to explain why the examined the activity and localization of p-MAPK.

Response: Thank you very much for your suggestion. We examined MAPK due to its classic roles on spindle organization during oocyte meiosis. We added the rational speculation for this at L312-315.

Could the authors reasonably explain why HDAC6 expression decreased and the expression of NAT10 increased in the MO injection group?

Response: Thank you very much for your question. Acetylated tubulin is analyzed as an indicator for microtubule stability. NAT10 (acetylation-related enzyme) and HDAC6 (deacetylation-related enzymes) have been reported to affect microtubule stability by regulating tubulin acetylation

during meiosis. In our results KIFC1 depletion induced raised acetylation level of tubulin. Increased NAT10 level and decreased HDAC6 level in KIFC1 knockdown oocytes could confirm the increased tubulin acetylation level for microtubule stability.

Why the authors studied the effects of KIFC1 depletion on ER?

Response: Thank you very much for your question. We did this to figure out how a microtubule motor protein is required for actin-dependent spindle migration. Actin nucleators are demonstrated associated with ER, which is related to the spindle in a microtubule-dependent manner. The most obvious hypothesis is that there is a defect in association of ER with the spindle in KIF1C knockdown. Moreover, the spindle-peripheral FMN2 nucleates short actin bundles from vesicles derived from the ER. We showed that KIFC1 depletion affected ER distribution, indicating that KIFC1 might disrupt association of ER with the spindle, which further affected the distribution of actin nucleators and actin-based spindle migration in mouse oocytes.

When both KIFC1 activity was inhibited or depleted by MO injection, there were still a proportion of oocytes extruded the polar bodies. What is the reason? KIFC1 regulated both spindle formation and migration. Is there over-sized polar body?

Response: Thank you very much for your questions. Yes, part of oocytes could extrude the polar bodies, and we think that this is due to the individual difference of oocytes. Even knock out approach could not disturb the maturation of all oocytes, and the inhibitor doses were determined to not induce oocyte death. We did not find big polar bodies, and only polar body extrusion defect was detected in KIFC1-MO oocytes. Big polar body was due to the failure of spindle migration and successful cytokinesis, while in our results cortical actin which was in charge of cytokinesis was also affected.

The figures are in good quality in general. But in Fig 4H and 6H, the loading control actin levels differ in different groups.

Response: Thank you very much for your remind. We changed the images with better quality bands to verify our results.

The language needs major improvement.

Response: Thank you very much for your suggestion. We totally re-checked the whole manuscript and revised the grammar and typo mistakes to improve our writing.

Reviewer 2
*Actually, kinesin superfamily of proteins (KIFs) have important roles in chromosome separation, microtubule dynamics, spindle formation, cytokinesis, and cell cycle progression. There were reported that Kif5B, Kif1B, Kif4, Kif11, Kif20A, Kif2A are all essential for normal oocyte meiosis in mouse oocytes including GVBD, spindle formation, PB extrusion (Mailhes et al., 2004; Gui and Homer, 2012; Kidane et al., 2013; Zhang et al., 2014; Chen et al., 2016; Kong et al., 2016; Camlin et al., 2017). Importantly, KIFC1 proteins involve in regulating DNA synthesis in S phase, and chromatin maintenance in not only mitosis, but also meiosis such as: KIFC1 is essential for normal spermatogenesis (Hao et al. 2019) or Spindle microtubules-associated protein HSET/KIFC1 labelling along the spindle was reported in mouse oocytes (Yang et al. 2020). Therefore, the author should discuss the result compared to KIFs related to meiosis in mouse oocyte (For example: KIFC1 was reported in mouse oocytes by Yang et al. 2020 was not cited). Because the present result 1 and 2 mention about localization of KIFC1 at all stages of oocyte maturation and KIFC1 was essential for mouse oocyte meiotic maturation.

Response: Thank you very much for your positive comments and questions. Yes, KIFs widely involved into multiple cellular processes, and KIFC1 expression was reported to be altered in BPA-treated and aged oocytes. As suggested we added the discussion about other KIFs and the related KIFC1 information to enrich our story. We also compared our results with other KIFs related to meiosis in mouse oocyte to highlight the novelty and meaning of our work.

*The author reported that involvement of Kif4a in spindle formation and chromosome segregation in mouse oocytes in previous report (2018). The present result 3 show that KIFC1 regulates spindle formation and chromosome alignment in mouse oocytes meiosis. It seems same function between Kif4a and KIFC1 in your research?
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Response: Thank you very much for your questions and the attention to our recent work. 1) As you mentioned above, KIFs have important roles in chromosome separation, microtubule dynamics, spindle formation, cytokinesis, and cell cycle progression both in mitosis and meiosis, while our recent studies focused on the roles of kinesin proteins in female meiosis such as Kif4a, Kif18a, Kif11 and Kif17. Previous findings together with our results showed that these kinesins did share conserved and similar roles in difference models. Their similar functions on microtubule dynamics and spindle function may be due to the high conserved domains of kinesin protein. Kinesins bind to cargos via their variable tail regions, and move the complex to the destination with the globular motor domain in head. Thus, some acetylation related enzymes may be the cargos of these KIFs on microtubules. 2) For the relationship between these kinesins, at least there was no report on the relation between KIFC1 and Kif18a. We do not think that kinesins are associated with each other for their functions, instead, as we mentioned above, this might be due to its conserved domain. 3) Although in mitosis some kinesins could regulate actin assembly, the roles of KIFC1 on actin assembly was reported for the first time in our study, in addition to the typical microtubule-related functions in oocytes. We believe that besides Kif17 there will be other kinesins which also regulate actin dynamics in oocyte meiosis.

*Because the authors have some published papers related to KIFs, the authors should make more clearer about regulation of KIFs during oocyte meiotic maturation to reader understand the specific function of KIFs during oocyte meiosis. The author can make graphical abstract about KIFs during oocyte meiotic maturation.

Response: Thank you very much for your suggestion. We enriched our discussion to relate the roles of other KIFs in oocytes. In fact, a relative review on the roles of kinesins in meiosis was published recently (Camlin NJ et al., 2017, Hum Reprod Update.), which visually illustrate the roles of KIFs during oocyte meiotic maturation, and we cited it in the manuscript. Since our study only focused on KIFC1, we think that it may be not appropriate to include other KIFs in our present research paper.

*On the other hand, some points in the article need additional information: the meaning of Nocodazole and Taxol treatment. The author should also make a clarification of using the concentration of AZ82 and K1FC2-MO in this study. Which factor the author based on to choose this concentration and whether increasing the treatment dose can completely inhibit the polar body extrusion as the results only show the partial effects of K1FC1 knock-down.

Response: Thank you very much for your suggestion. Yes, for nocodazole and taxol treatment Reviewer 1 also raised this issue, and we added this rational information in the Results (L257-260). The concentration of AZ82 was based on previous reports and our dose test, and the MO concentration was followed by the product instructions, and we added this information in Methods part. The concentrations we adopted was to ensure statistical significantly difference, and extremely high dose inhibitor treatment could completely inhibit oocyte maturation but we think that high dose will have cytotoxicity on oocytes. For knock down approach, we do not think that knock down could completely inhibit the polar body extrusion since even knock out approach there was about 10% remain expression of target protein, and there was individual difference for oocytes.

*Some minor points in this study that the author should be considering. In figure 3A, there should be staining of K1FC1 to clarify the effects of AZ82 and knock-down treatment. In figure 3D, the picture of p-MAPK in control-MO looks the same as AZ82 and knock-down treatment, yet the result showed that they were different.

Response: Thank you very much for your remind. For KIFC1 staining on AZ82 and knock down treatment, we had knock down efficiency test by western blot and polar bod extrusion data to confirm their effects. The inhibitor AZ82 specifically suppressed KIFC1 activity rather than affected its expression level, so there will be no difference for KIFC1 antibody staining; for knock down, we think that western blot results would be more reliable, since fluorescence staining is mainly used for the localization pattern instead of protein level examination. We replaced a more appropriate and typical picture for control-MO group in figure 3D.

*Please correct Fig number Fig. 1D to 1E and Fig. 1E to 1D.

Response: Thank you very much for your remind. We re-organized this.

Second decision letter

MS ID#: DEVELOP/2021/200231

MS TITLE: Kinesin motor KIFC1 is required for tubulin acetylation and actin-dependent spindle migration in mouse oocyte meiosis

AUTHORS: Meng-Meng Shan, Yuan-Jing Zou, Zhen-Nan Pan, Hao-Lin Zhang, Yi Xu, Jia-Qian Ju, and Shao-Chen Sun
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 finds an important role of kinesin KIFC1 in tubulin acetylation-
based spindle integrity and regulation of actin dynamics for spindle migration in meiotic oocyte.

Comments for the author

The revised manuscript well addressed my concerns raised in the first review.
Minor comments: language improvement by a specilized proof-reading company or an expert in recommended.

Reviewer 2

Advance summary and potential significance to field

- Novel finding of KIFC1 in oocyte meiosis – Previous study have only reported roles of KIFC1 in mitosis and meiosis of spermatocyte.
- Method: applying combination of several techniques for detection of one results (extremely powerful results)
- Clear figure: showed clear relationship among KIFC1 ,alpha-tubulin , and spindle migration.

Comments for the author

The authors have extensively revised the manuscript according to the reviewer's suggestions.