Basic reporting:

In this manuscript, Xie *et al* report the role of OLA1, a member of the GTPase protein family in oocyte meiosis. The authors show that OLA1 can regulate germinal vesicle breakdown (GVBD), spindle assembly and spindle assembly checkpoint (SAC) activation during oocyte meiosis. Transient siRNA knockdown of OLA1 is associated with defects in spindle assembly and SAC inactivation during meiosis. While the authors validate their findings in a clear manner, a few points of concern are mentioned below:

Overall the results support the hypothesis put forward by the authors, but the manuscript needs careful proofreading. The authors report the role of OLA1 in oocyte meiosis. However, there is not sufficient mention or explanation of the oocyte meiotic cycle in the Introduction section. A brief description of the oocyte cycle should be described, explaining the process of GVBD and polar body extrusion (PBE). Sufficient references and background context is provided otherwise.

Earlier studies have already shown that OLA1 is implicated in centrosome amplification and astral microtubule formation during mitosis, associated with spindle defects. The role of OLA1 in meiosis, exhibiting spindle assembly defects would have been predictable to some extent. Thus, in terms of novelty, this manuscript might require additional mechanisms to strengthen their observations.

There are several grammatical errors throughout the manuscript which if corrected will enhance the quality of this article considerably.

Line 59 “Mammalian gametes are yield” is incorrect grammar.

Line 66 “normal progress of oocyte meiosis” should be rephrased as “normal progression of oocyte meiosis”.

Line 71, 216, 263: “premature of chromosome segregation” is again incorrect, should be rephrased as “premature chromosome segregation”.

Line 73 use “established instead of “amended”.

The transition from line 76 to line 77 has no connecting link. The authors might want to atleast write a sentence highlighting why they were interested to study the role of OLA1 in meiosis after describing meiosis in line 76.

Lines 80-84 is directly copied from the reference Matsuzawa *et al* 2014. The authors need to rephrase the observation in their own language if they are citing a reference.

Line 87, 189, 255: “interactive factor/protein” is not sounding correct. The author means to say OLA1 is an interacting partner, not interactive.

Line 91: “whether OLA1 involve in meiosis” is again grammatically incorrect. It should be “whether OLA is involved in meiosis”

Line 108: “mice firstly intraperitoneally injected” should be “mice were intraperitoneally injected”.

Line 116: “wild type” instead of “wide type”

Line 117: “To reassemble” instead of “To reassembly”

Line 167: “oocytes were thoroughly washed out of nocodazole” is not the correct representation of the sentence. It should be “nocodazole was washed out”.

In the Results section, where the authors describe that silencing OLA1 abrogated GVBD and subsequently PBE, they should describe how did they assess the GVBD and PBE rate?

The transition from line 213 to the subsequent section describing SAC inactivation upon OLA1 knockdown is missing. The authors should begin this section by mentioning why are they looking at SAC activation and its relevance to the context.

Line 228: “processes” instead of “progresses”

Line 232: “cell cycle symbols” should be replaced with some appropriate word. Maybe “GVBD and PBE are characteristic features/hallmarks of meiotic progression”.

Line 244: “During the time spindle assembly” should be “During the time of spindle assembly”.

Line 250-251 needs grammatical error correction and rephrasing.

Line 263: “mouse oocyte” instead of “muse oocyte”

Line 264: “Taken” instead of “Token”.

Line 266-267 needs rephrasing.

The Discussion section of the article is redundant and is simply states the observations reported in the Results section. An appropriate explanation of the phenotypes reported needs to be provided.

The raw data showing the Western blots of OLA1 expression during meiosis needs labelling of what the lanes correspond to.

Experimental design:

The authors validate their hypothesis by relevant experiments. They report several significant phenotypes that are characteristic of defective spindle assembly during meiosis. The images shown are representative of the defects mentioned in the article. A few suggestions that the authors might consider are mentioned below:

The authors may want to conduct some colocalization studies with known spindle proteins in mouse oocytes during meiosis. This would further validate that OLA1 colocalizes with the meiotic spindle (Figure 1A).

The blot showing OLA1 expression (Figure 1B) during meiosis should be probed with stage-speficic meiosis markers to validate that particle stage. For example, SCYP3 antibody can be used as a marker for meiosis. Other proteins that are expressed during GV, GVBD, MI and MII should be probed along with OLA1 to validate the corresponding stage of meiosis.

The authors should mention the experimental procedure to measure the GVBD and PBE rate (Figure2).

This article shows several phenotypic defects on spindle assembly upon OLA1 knockdown. However, the authors do not show any rescue experiments by expressing OLA1 to show that the defects can be restored.

The authors show multipolar spindles and chromosome misalignment as a readout of aberrant spindle assembly (Figure 3). They might want to provide some insight into spindle orientation upon OLA1 knockdown by measuring the spindle angle relative to the substratum. Typically, in control cells, the spindle angle should be close to zero, as they are aligned parallel to the substrate. However, during misorientation, there is an increase in the spindle angle.

In the experiment showing anaphase inset in mouse oocytes, was the population synchronized? (Figure 4) It is better to synchronize the cells and then study the meiotic progression to get uniform data set. A flow cytometric analysis showing the progression of a synchronized population of mouse oocytes should generate data that describes anaphase onset better.

Another general question that the authors may want to comment on would be whether the function of OLA1 in maintaining spindle assembly is dependent on the BARD1/BRCA1 complex or it acts independently? It has been shown that OLA1 binds to BARD1/BRCA1 complex to regulate centrosome amplification during mitosis. Mutants of OLA1 that are unable to bind to this complex do not act efficiently to control centrosome number. Thus, it may be worthwhile for the authors to explore the mechanism of action of OLA1 that underlies the defects observed during meiotic spindle assembly.

Validity of the findings:

The authors show convincing defects in spindle assembly during meiosis upon silencing OLA1. It is recommended to conduct rescue experiments to check if the observed phenotypic defects can be restored upon OLA1 expression. This will help in complementing he data further.

Also, it would be good if the authors explained the mechanism of action of OLA1. Since it has been reported that OLA1 functions together with BARD1/BRCA1 to control centrosome number, the authors could shed light on whether OLA1 acts cooperatively with this complex to drive spindle assembly. Also, it would be interesting to check if mutants that are not able to bind BARD1/BRCA1 can still exhibit these phenotypic defects.

General comments for the author:

This data shown in this manuscript is convincing. The authors report interesting data, validating the role of OLA1 in spindle assembly. The experiments performed are relevant and support the hypothesis well. However, some additional experiments, like the rescue of the observed defects in spindle assembly upon OLA1 restoration might be important. Some sections of the manuscript particularly the Discussion seemed redundant and can be rephrased to explain the results better. The Introduction section should provide some background on the meiotic cycle in oocytes. Careful proofreading is required before the final submission as this manuscript has several typos and grammatical errors.

Signed by: Ishani Dasgupta