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Reviewer A

This interesting study demonstrates that KIF23 is a potential oncogene in triple-negative breast cancer, MiR-195-5P is downregulated in triple negative breast cancer tissues and suppresses KIF23 expression. Knockdown of KIF23 by siRNA inhibited proliferation and migration of triple-negative breast cancer cell lines MDA-MB-231 and BT549. Albeit, I consider these findings provide insight into how KIF23 regulates cancer development, and these data as potentially valuable to guide further research into the role of KIF23 in cancer therapies. I still have some minor suggestions for the authors.

1. The author used MTT assay for short term sensitivity and proliferation analysis, is this possible that the author can get similar results in long term proliferation analysis such as the soft agar assay.

Reply: Thanks for your professional advice. In addition to CCK8 assay, the colony formation assay was conducted in our research (see Page 7, line 13-21, and Fig. 2c). The colony formation is a long term proliferation analysis and get accordant results comparing with CCK8 assay.

2. There are some grammatical mistakes in the manuscripts, I suggest the author review the English grammar to correct minor mistakes. For example, in the abstract (Line 5): …. were conducted to analysis expression…"analysis" should be corrected as "analyze".

Reply: Thanks for your correction. We have modified our text as advised (see Page 2, line 12).

3. In Result (Line 15), 293T cells were transfected with miR-195-5p mimics and wildtype KIF23-3'UTR or "mutanted"...; "mutanted" should be corrected as "mutated".

Reply: Thanks for your correction. We have modified our text as advised (see Page 13, line 2).

Reviewer B

As the manuscript title stated, Jian et al. claimed that KIF23 promotes triple negative breast cancer (TNBC) through activating Epithelial Mesenchymal Transition (EMT). For this, they conducted a series of experiments as follows;

(1) Effects of KIF23 knockdown on TNBC cells were assessed by cell proliferation and migration assays.

(2) Mechanisms of action of KIF23 in TNBC cells were investigated by western blotting assays (WB) to see the effects of KIF23 KD on the expression levels of some EMT biomarkers, such as E-cadherin and N-cadherin as well as vimentin.

(3) A role of miR-195-5p in KIF23 expression was investigated by the dual luciferase assay to see whether miR-195-5p recognizes KIF23 mRNA.

However, the experiments performed by the authors appeared to be incomplete to clarify the mechanisms of action of KIF23 in TNBC cells and some experiments suffered from substantial technical flaws that ought to be rectified.

Major concerns

A) Because the experimental read-out to characterize the change of KIF23 mRNA expression levels in this manuscript is essentially based on RT-PCR technique, the reliability of this technique must be described, controlled and backed up by another technique. In this context, two publications may be helpful:

(1) Haddad et al. (2007): Potential pitfalls in the accuracy of analysis of natural sense-antisense RNA pairs by reverse transcription-PCR. BMC Biotechnology, 4 (7): 21.

(2) Houseley and Tollervey (2010): Apparent non-canonical trans-splicing is generated by reverse transcriptase in vitro. PLoS One, 18 (5): e12271.

Accordingly, multiple RTs should be tried in initial experiments, including primer-less RT followed by normal PCR to exclude self-priming. Furthermore, a northern blot is required with sense and antisense RNA probes and polyA+/- RNAs.

Reply: Thanks for your professional advice. As a matter of fact, in order to ensure the specificity of KIF23 primer, the dissociation curve was analyzed in qPCR. As our result showed (see Supplementary material, Fig. S4), the single peak curve indicated primer of KIF23 in our research was specific.

A) In Fig. 2d, the scratched lesions were narrowed at 24h in both NC and KIF23-si1 cells when compared to those observed at 0h. These results thus suggested that the cell culture condition (2% FCS) applied to the wound healing assay did allow

TNBC cells to proliferate in addition to migrate into the lesions. The authors should therefore titrate FCS concentrations prior to the assay, with which TNBC cells do not proliferate but migrate to the lesions.

Reply: Thanks for your professional advice. In order to analyze the migration change of TNBC cells after knockdown of KIF23, both wound healing assay and transwell assay were conducted in our research, we combined these results to analyze migration ability change of TNBC cells.

A) Fig. 3 showed that KIF23 KD resulted in the change of protein expression levels of Epithelial-Mesenchymal Transition (EMT) biomarkers: such as E-cadherin and N-cadherin as well as vimentin. These results suggested that KIF23 may positively affect EMT as claimed by the manuscript title.
However, in order to verify that the WB results presented in Fig. 3 were not due to off-target effects by siRNA-dependent gene silencing technique and that the EMT pathway is necessary and sufficient for TNBC cell proliferation and motility, the authors are requested to employ additional complementary approaches as follows;
(1) See whether re-expression of wild-type KIF23 in KIF23 knockdown TNBC cells promotes EMT.
(2) See whether knockdown of both N-cadherin and vimentin and overexpression of E-cadherin in TNBC cells suppress cell proliferation and motility.
(3) See whether overexpression of both N-cadherin and vimentin and E-cadherin knockdown in TNBC cells promote cell proliferation and motility.
Collectively, these experiments enable the authors to claim that EMT pathway mediates KIF23-dependent tumor cell proliferation and motility.

Reply: Thanks for your professional advice. We transfected MDA-MB-231 and BT549 cells with KIF23 plasmid, the Western blot results showed overexpression of KIF23 promoted EMT (see Page 11, line 21-22, Page 12, line 1-2, and Fig. 3a). N-cadherin is a marker of EMT, we analyzed proliferation and migration change of TNBC cells by colony formation and transwell assay after knockdown of N-cadherin or overexpression of N-cadherin. As our results showed, knockdown of N-cadherin suppressed proliferation and migration of TNBC cells, while overexpression of N-cadherin promoted proliferation and migration of TNBC cells (see Page 12, line 2-4, and Fig. 3b,3c). Collectively, these results indicated KIF23 influenced proliferation and migration of TNBC cells through EMT pathway.

D) The authors claim that miR-195-5p acts as tumor suppressor by regulating KIF23 mRNA levels. They validated this hypothesis by dual luciferase assay.

In order to further verify their claim in more physiological condition, it might be an idea to employ antagomiR (synthetic oligoribonucleotide complementary to miR-195-5p seed sequence) to knock down miR-195-5p in TNBC cells and see the changes of KIF23 mRNA expressions and the changes of cell proliferation and motility accordingly.

Reply: Thanks for your professional advice. We added some data in our research (see Page 13, line 5-8, Fig. 4e). As our results showed, miR-195-5p could inhibit proliferation and migration of TNBC cells. Because of limited time and lack of available miR-195-5p inhibitor, we just analyzed function of miR-195-5p mimics in TNBC cells.

A) It would help enormously if the KIF23 genomic locus, including introns and locations of both PCR primer pair and siRNA1-3 are shown, and if schematic drawings of all plasmid inserts used are shown in Supplementary Materials.

Reply: Thanks for your professional advice. We added the KIF23 siRNA1-3 sequence in our research (see Page 7, line 21-22, Page 8, line 1-6).

Minor points

A) Fig. S1 legend title: expression of VGLL4 in major breast cancer subtypes should be read as expression of KIF23 in major breast cancer subtypes.

Reply: Thanks for your correction. We have modified our text as advised (see Page 19, line 2).

B) In Fig. 1a, please state which color (red or green) corresponds to TNBC tissue or paired paracancer tissues.

Reply: Thanks for your advice. We have modified our text as advised (see Page 19, line 10-13, Page 12, line 1-2).

C) Figs. 1b and 2d, e require scale bars.

Reply: Thanks for your advice. We annotated the magnification of microscope (see Page 20, line8, 9, 16, Page 21, line 5)

D) In Fig. 3a, c, please provide quantification results of each protein band intensities to help to quantify the WB results, since these WB bands were scanned with Odyssey.

Reply: Thanks for your advice. We have modified our text as advised (see Fig. 4b)

E) There are a large number of errors in English. The authors should consult a professional proof-reader(s) for their English prior to resubmitting amended manuscript. The errors I noticed, but not limited to, are listed below;

(1) p4, lines1-2: relative patients should be read as patient relatives.

(2) p4, line 8: is ‘storied’ ‘stored’?

(3) p10, lines 3-10 consist of one sentence. This should be divided to several sentences.

(4) p11, line 1, what does ‘famous gene’ mean? Does this term mean ‘well-studied’ or something else?

Reply: Thanks for your correction. We have modified our text as advised (see page 4, line15; Page 5, line 2; Page 12, line8-18; Page 13, line 15).