Author’s response to reviews

Title: CCNE1 Amplification Is Associated with Poor Prognosis in Patients with Triple Negative Breast Cancer

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Author’s response to reviews:

Dear Dr. Chakrabarty,

Thank you for your thorough review of our manuscript BCAN-D-18-02443 titled “CCNE1 Amplification Is Associated with Poor Prognosis in Patients with Triple Negative Breast Cancer” by Zi-Ming Zhao, PhD; Susan E. Yost, PhD; Katherine E. Hutchinson, PhD; Sierra Min Li, PhD; Yate-Ching Yuan, PhD; Javad Noorbakhsh; Zheng Liu, PhD; Charles Warden; Radia M. Johnson; Xiwei Wu, PhD; Jeffrey Chuang, PhD; Yuan Yuan, MD, PhD

We made revisions according to Dr. Khattar and Dr. Chakraborti’s recommendations, and hope we have satisfactorily addressed all comments. Our detailed descriptions of the changes are listed below.
We requested a change in authorship and submitted a signed “Request for Change in Authorship” form on 12/10/18 for Zi-Ming Zhao as first author and Yuan Yuan as last author.

Reviewer 1, Anindita Chakrabarty, PhD: This is a very nice study aiming to uncover the genetic heterogeneity of paired primary and metastatic TNBC with two independent sample sets, pilot and discovery-TNBC. This study revealed coexistence of CCNE1 and TPX2 amplification in metastatic lesions. CCNE1 amplification was further associated with poor overall survival and possible mechanism of chemo-resistance. Even though this was small-scale study it was thoroughly done. Recommendation: accept.

Thank you for your kind review.

Reviewer 2, Ekta Khattar: Authors have suggested that this is a hypothesis generating study and needs further verification using larger datasets. Overall study is clearly described.

Minor Revision Point:

1. Add discussion about how mechanistically cycle E amplification might be helping in developing chemo resistance in cancers.

We thank the reviewer for the very helpful suggestion. We have revised the manuscript by adding discussion about the role of CCNE1 amplification in chemo-resistance (page 12 line 274).

Cyclin E and its associated CDK2 are essential for cellular progression through the G1 phase of the cell cycle and initiation of DNA replication. CCNE1 amplification induces chromosome instability through persistent DNA replication and centrosome duplication [43-45]. CCNE1, along with its catalytic subunit CDK2, plays a critical role in cell cycle regulation to assure precise control of DNA replication, chromosome segregation and the G1 to S-phase transition [11, 12].

Reviewer 3, Gopal Chakraborti: The article "CCNE1 Amplification Is Associated with Poor Prognosis in Patients with Triple Negative Breast Cancer" was well scientific written research paper by the authors. The work has good impact on TNBC patients for targeted therapy suppressing CCNE1 for the betterment of the prognosis of TNBC patients. However, a few comments and additional work are suggested to validate the hypothesis/ findings

Comments

1. The supplement figure showed the high expression of CCNE1 and TPX2 in compared to normal and TNBC patients. However, authors showed only CCNE1 expressions (mRNA
profiling) in the figure 4. Why it is so. Is there any correlation of CCNE1 and TPX2 in TNBC patients in mRNA level? Please address this question.

We thank the reviewer for the insightful comments.

We have previously performed analysis of TPX2 mRNA expression and its association with survival. No association was identified.

In Figure 3C (METABRIC) and 3D (TCGA) recopied below, clear correlation between CCNE1 and TPX2 mRNA expression were identified and shown here.

Both our “Pilot-TNBC” cohort (n= 22) and “Discovery-TNBC” cohort (n = 95) showed strong correlation between CCNE1 and TPX2 mRNA expression, both with p < 0.001. The description was added to page 9 Line 204 and Supplemental Figure 2 was added:

Furthermore, CCNE1 is significantly co-overexpressed with TPX2 in “Pilot-TNBC” and “Discovery-TNBC” cohort (p <0.001) (Supplemental Figure 2)

2. Why TXP2 is not included in the title of the article?

In our study, there was no association between TPX2 and patients’ clinical outcome; therefore, we thought TPX2 was not relevant to our key findings.

3. The protein profiling like ELISA or Western blot should be done for the genes mentioned in figure 1 e.g. p53, Pi3K, mTOR etc.

Figure 1 showed no significant genomic landscape shift between paired primary and metastatic TNBCs in our study, and currently we do not have sufficient tumor samples left for protein analysis. In addition, the METABRIC and TCGA database have only limited proteomic data (RPPA) available for comprehensive analysis. However, we recognize the value of studying proteomic alterations in paired TNBC tumors and this important question will be addressed by future studies.

4. Other changes

The Acknowledgments have been revised to include NIH P30CA033572.

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mRNA expression data has been added in Supplemental Table 1(Pilot-TNBC) and Table 2 (Discovery-TNBC). Genomic alteration data has been added in Supplemental Table 2 (Pilot-TNBC).

- Declarations
- Ethics approval and consent to participate
- Consent to publish
- Availability of data and materials
- Competing interests
- Funding
- Authors' Contributions
- Acknowledgements
- Authors' Information

All Declarations are included.

On behalf of the authors, thank you for your consideration of our manuscript.
Warm Regards,

Yuan Yuan MD PhD