Peer Review File

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The authors examine expression of the transcription co-activator CTBP2 in relation to CP sensitivity and patient outcomes in LUAD. High expression of CTBP2 correlated with worse outcomes in NSCLC patients. NSCLC cells were divided into high and low expression groups and correlated with CP IC50 values from the GDSC database. The findings are that CTBP2 is elevated in about half of the relatively resistant NSCLC cell lines. CTBP2 expression was elevated in an A549 derivative that is CP resistant. Knockdown of CTBP2 in this derivative enhanced CP sensitivity associated with changes in apoptotic proteins and mitochondrial membrane potential. Authors conclude CTBP2 contributes to CP resistance in NSCLC.
Main concerns:
1) In Fig 3 at least half (6/12) CTBP2-high cell lines had low IC50 values comparable to CTBP2-low cells. Further, IC50 values for the CTBP2-high cells varied despite CTBP2 expressions being comparable. There is not a clear relationship between CTBP2 levels and CP sensitivity. Minimally the authors need to point out these observations and indicate that the relationship between CTBP2 expression and CP sensitivity IC50 values is not absolute.

Reply： Thank you for bringing up your observations regarding Figure 3 in our manuscript. We appreciate your attention to detail and agree that there is a noteworthy finding in the data.
As you correctly pointed out, at least half (6/12) of the CTBP2-high cell lines displayed low IC50 values, comparable to the CTBP2-low cells. Interestingly, despite having comparable CTBP2 expressions, the IC50 values varied among the CTBP2-high cells. This observation suggests that there is not a clear and absolute relationship between CTBP2 levels and CP sensitivity.

We acknowledge that these findings are important and should be highlighted in our manuscript. We will make the necessary revisions to explicitly mention these observations and emphasize that the relationship between CTBP2 expression and CP sensitivity, as reflected by the IC50 values, is not absolute. we have modified our text as advised (Page 7, line 263-267, and marked in red.
Changes in the text: Page 7, line 263-267. As shown in Fig. 3C-D, the CP IC50 values in high-expression CTBP2 cells were higher than that in low-expression cells. However, CTBP2 high expression cell lines had low IC50 values compared to CTBP2 low expression cells, the relationship between CTBP2 expression and CP sensitivity is not absolute.

2) The extent of CTBP2 knockdown by shRNAs is not very striking in Figs 4 and 5. Perhaps complete knockdown of CTBP2 is not viable? Have the authors tried a transient knockdown by siRNA? Would it also sensitize these cells to CP while

showing greater knockdown?

Reply： thank you for your comments.Regarding the extent of CTBP2 knockdown shown in Figures 4 and 5, we understand your observation that it may not appear very striking. We agree that complete knockdown of CTBP2 might not be viable in the experimental conditions used in our study. However, it is important to note that even partial knockdown of CTBP2 can have significant functional consequences, as demonstrated by the results presented in our paper.

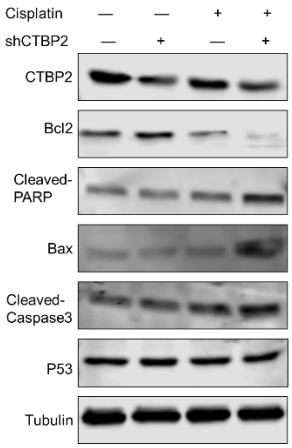
To address your question about transient knockdown using siRNA, we have indeed explored this approach in our preliminary experiments. While we did observe a greater knockdown efficiency with siRNA, we found that the effects were transient and not sustained over a longer period. This limited the ability to assess the long-term effects of CTBP2 knockdown on cellular sensitivity to CP.

Nevertheless, we appreciate your suggestion and agree that further investigation into transient knockdown using siRNA could provide valuable insights. We will consider incorporating this approach in our future studies to explore the potential for enhanced knockdown efficiency and its impact on cellular response to CP.

Changes in the text: None.

3) Fig 5 shows CTBP2 shRNA increased CP induced apoptosis in these A549 resistant cells associated with changes in apoptotic proteins and increased cleaved PARP and caspase 3. A549 are p53 WT. Is p53 induced more by CP in the CTBP2 knockdown cells, and does it contribute to the CTBP2 knockdown effect?

Reply： Thank you for your comments. Considering this, your query regarding the induction of p53 in CTBP2 knockdown cells upon CP treatment and its potential contribution to the observed effect is indeed intriguing. To address this, further investigation and experimentation would be required. Based on bioinformatics analysis, CTBP2 expression in patients with TP53 mutation was no different in contrast with that in patients with non-TP53 mutation. Furthermore, we detected the p53 expression by western blot. The results showed that P53 expression is not change in CTBP2 knockdown or cisplatin treatment. Therefore, P53 does not contribute to the CTBP2 knockdown effect.



Changes in the text: None.

4) Fig 2 shows the relationship between CTBP2 expression and patient outcomes. The overall notion is that high CTBP2 expression may cause lower response to cisplatin-based chemotherapy and subsequently worse outcomes in patients. Were all these patients in Fig 2 treated with cisplatin? Is it possible to separate out the CP-treated ones to ask if CTBP2 expression correlates with outcomes in CP-treated patients?

Reply： thank you for your comments. Regarding your question, it is not explicitly mentioned whether all the patients in Fig 2 were treated with cisplatin. it would be beneficial to separate out the cisplatin-treated patients to investigate whether CTBP2 expression correlates with outcomes specifically in this subgroup. By doing so, we could gain further insights into the impact of CTBP2 expression on the response to cisplatin-based chemotherapy. However, based on Kaplan-Meier Plotter database, there did not separate out the patients treated with CP. Therefore, we can only provide an overall survival rate of CTBP2 in LUAD.

Changes in the text: none.

Other concerns:

5) Fig 1A; label red tumor and gray normal

Reply： yes, have done.

Changes in the text: Page 13, line 448 has been modified. red tumor and gray normal

6) Fig 1C; they are all elevated vs normal, it is not clear however that CTBP expression increases with increased stage. Writing implies that it is. Discussion text for example says CTBP2 expression correlated with stages 1-4. Can the authors clarify this?

Reply: thank you for your comments. Indeed, CTBP2 is high expression in all stage compared to normal group. In further analysis, we find that CTBP2 levels of stage3 and stage4 are higher than that in stage1. Therefore, the CTBP2 expression level can

be used as a basis for staging of LUAD.
Changes in the text: Page 7, line 233 to 237 has been modified. the results obtained from the UALCAN data revealed that the expression of CTBP2 was higher in grade 1 to grade 4 compared to normal tissues, furthermore, the expression of CTBP2 in grade3 and grade4 increased compared to that in grade1, illustrating that CTBP2 was associated with the LUAD grade.

7) Fig 1D: is the normal sample here normal tissues from a specific race? Should the writing indicate CTBP2 was elevated in LUAD from these races vs race-matched normal control tissues?
Reply: thank you for your comments. the normal sample is not a specific race.
Changes in the text: Page7, line 238 has been modified. All race normal.

8) Fig 1G: the labeling of this figure implies CTBP2 is elevated in nodal vs primary, but maybe not different between N0, 1, 2, 3? Is this correct? If yes, the writing needs to be more clear and a description of what N0,1,2,3 means.
Reply: thank you for your comments.
Changes in the text: Page 7, line 241 to 242 has been modified. CTBP2 was significantly overexpressed in metastatic foci compared to normal tissues.
Page 13, line 450 to 452 has been modified. N0: No regional lymph node metastasis; N1: Metastases in 1 to 3 axillary lymph nodes; N2: Metastases in 4 to 9 axillary lymph nodes; N3: Metastases in 10 or more axillary lymph nodes

9) Fig 2: define FPS and PPS on line 236 and Fig 2 legend. Legend should also indicate total patient number (n) for each graph.
Reply: thank you for your advice. We will add the information.
Changes in the text: page 14, line 457 to 458 has been modified. CTBP2 overexpression is correlated to poor OS (A, n=719), FPS (B, n=125), and PPS (C, n=461) in patients with LUAD.

10) Fig 5B has error bars - how many experiments does this represent? Also, I don't think apoptosis "rate" is correct since the rate of apoptosis was not measured. I think just percent apoptosis is more correct.
Reply: thank you for your comments. We did apoptotic experiments repeated 3 times. And we accept your advice to correct the percent of apoptosis.
Changes in the text: page 17, figure 5 B has been modified.