Peer Review File

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Reviewer A

Cyclin O (CCNO) is a novel cyclin family protein containing a cyclin-like domain, which plays a role in cell cycle regulation. Recent research suggests that inhibition of CCNO leads to cell apoptosis in gastric cancer, cervical squamous cell carcinoma, and post-operative lung cancer. In the manuscript "Cyclin O promotes lung cancer progression and cetuximab resistance via cell cycle regulation and CDK13 interaction", authors examined whether CCNO is substantially expressed in LUAD and supports the malignant progression of the tumor phenotype.

Couple questions are required to be answered before it will be accepted.

(1) What were the correlations between cyclin, CDK and EGFR? Please state in the introduction.

Reply1: Accomplished

Changes in the text: The eukaryotic cell cycle is regulated by the temporal activation of different cyclin-dependent kinase (CDK)/cyclin complexes. Through these major transitions is mediated by sequential activation and inactivation of cyclin-dependent kinases (CDKs), which is frequently accompanied by phosphorylation of serine and threonine often occurs.[3]Meanwhile, the cyclin activation and the repression of cyclin-dependent kinase inhibitor proteins (CDKi) can regulated by *EGFR* signal pathway.[4]

(2) It was better to add related reference (DOI: 10.21037/tbcr-20-54) about the Cyclin-dependent kinase in the introduction.

Reply2: Didn't find it in SCI hub.

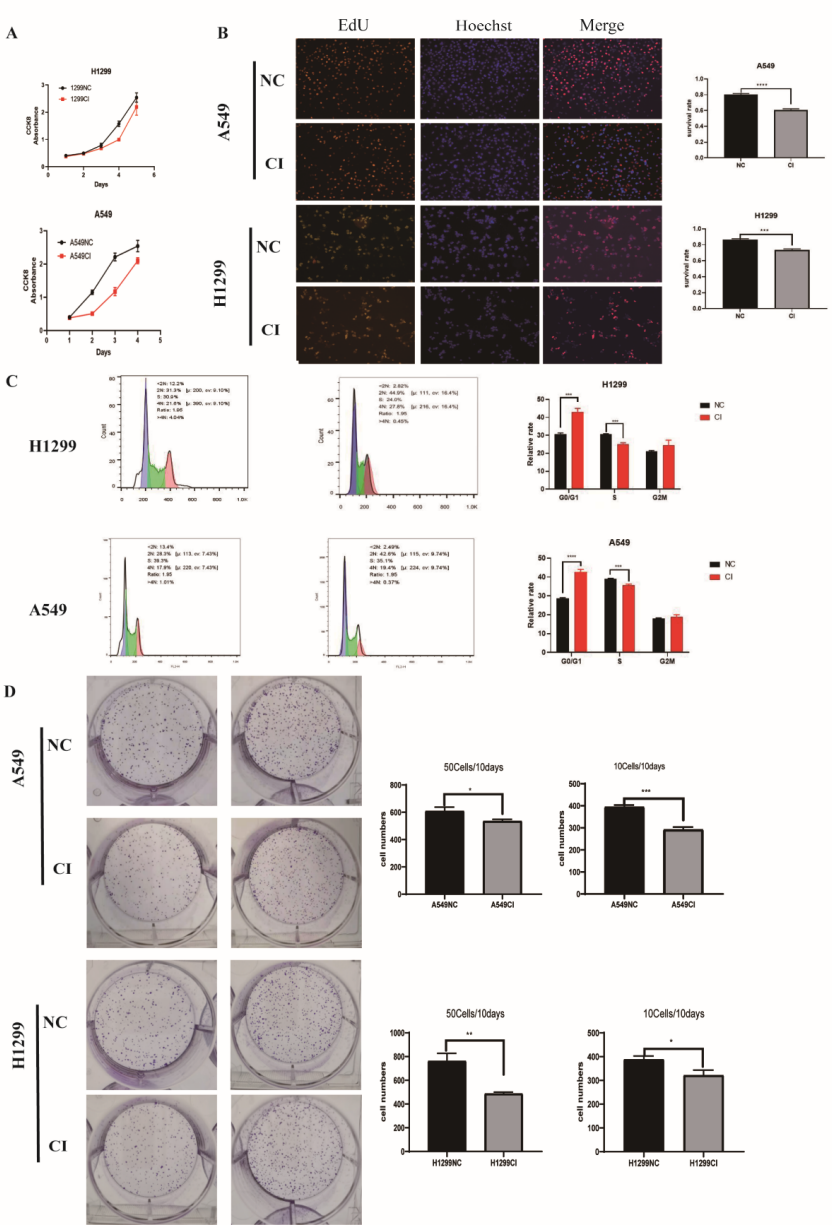
(3) Why to use BALB/c mice? Please state in the methods.

Reply 3: accomplished

BALB/c nude mice (male, 6–8 weeks old) were used for the animal experiments because of LUAD cell lines injection derived from human.

(4) In the figure 2B, the image was seemed to overlap an image. And mark the EdU and Hoechst.

Reply 4: No overlap. Accomplished.



(5) Why to obtain that CCNO expression was negatively correlated with cancer cell proliferation, migration, and invasion? Please state in the results.

Reply 5: Accomplished

At the beginning of our screening, CCNO, as an oncogene, showed its oncological effect in LUAD. CCNO showed its relationship with the survival and prognosis of LUAD patients in the database and clinical sample statistics. It is our preliminary exploration that CCNO can be used as a biomarker of LUAD.

(6) There were many grammar mistakes, and the writing was non-native like. Please check and revise.

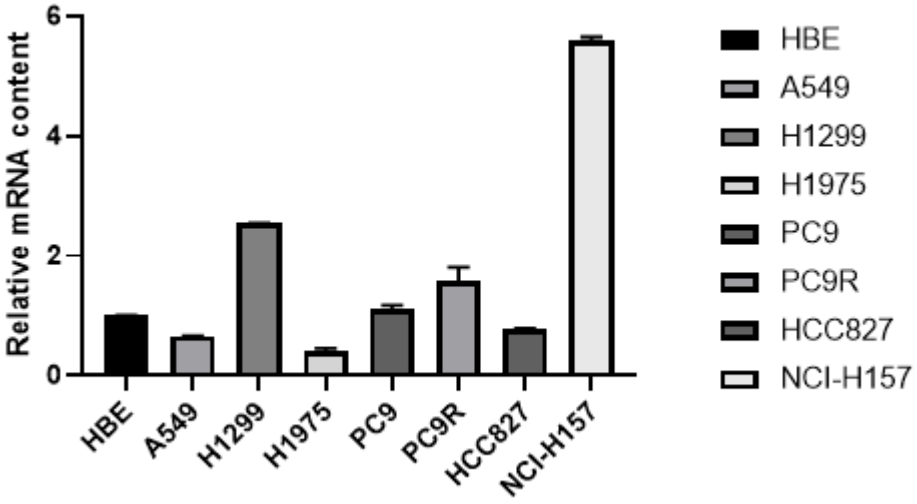
Reply6: Accomplished.

(7) Why to focus on CDK13? Please state in the discussion.

Reply7: Accomplished.

As is well known, the classic mode of action of cyclin is to bind to cyclin kinase in a dimeric form, activating downstream molecular signaling pathways through phosphorylation. A series of molecules found in the IP-MS.

(8) In Supplemental materials, the figure 1 should be replaced by a new.
Reply 8: Accomplished.



Reviewer B

1) First, the title did not indicate the other focus of this study, the prognostic role of CCNO.
   Reply 1: I suggest that CCNO promotes the progression of lung cancer, indicating a correlation with its prognosis

2) Second, the abstract needs further revisions. The background did not indicate the knowledge gap on CCNO in LUAD and the clinical needs for this research focus. The methods did not describe the clinical sample, the clinical data, and prognosis outcomes. The results need to quantify the findings by reporting statistics such as HR and correlation coefficients and accurate P values. It would be helpful if the authors have some comments for the clinical implications of the findings in the conclusion.
   Reply 2.1: the clinical sample, the clinical data, and prognosis outcomes have been described in result and table.
   Reply 2.2: Accomplished

3) Third, the introduction of the main text needs a detailed review on what has been known on the prognostic biomarkers, have comments on their limitations, clearly indicate the potential strengths of CCNO in comparison to these known biomarkers, and have comments on the knowledge gap and clinical relevance on the research focus on CCNO.
   Reply3: accomplished.
   Several proteins, cirRNA, and mRNA have been published to indicate their association with the prognosis of LUAD, but there is little further exploration of their molecular mechanisms.

At the same time, there is only a prompt and warning effect for lung adenocarcinoma patients, and there is no guiding significance for clinical treatment.

4) Fourth, in the methodology of the main text, please have an overview of the research procedures of this study, including the analysis on the prognostic role of CCNO and the part of animal experiment. The authors need to provide more data on the clinical sample, clinical factors collected, follow up procedures, and prognosis outcomes. In statistics, please ensure P<0.05 is two-sided. It is necessary to ascertain the independent prognostic role of CCNO before the animal study since the convincing and moderate-to-severe prognostic role is the prerequisite for the subsequent animal research.
Reply4: The admission criteria for LUAD patients were mentioned in the Table 1, and we continuously telephone follow-up to tracked the survival time of the patients for statistical analysis.

Reviewer C

1. Please check all abbreviations in the abstract and main text, such as below abbreviations in the abstract. All abbreviated terms should be full when they first appear.

Methods: The protein expression and signal transduction were detected by Western blot (WB) and immunohistochemistry (IHC). Overexpression or lacking *CCNO* stable cell lines were transfected with lentiviruses and selected with puromycin. The tumor behaviors of LUAD cells were assessed: cell proliferation by EdU staining and CCK8

Reply: accomplished.
Changes: The tumor behaviors of lung adenocarcinoma (LUAD)cells were assessed: cell proliferation by 5-Ethynyl-2'- deoxyuridine (EdU) staining and Cell counting kitting-8 (CCK8) assay.

2. The citation of references in your text is not in order. Please check the citation of reference 23; it should not appear behind references 24. Please revise.

signaling pathways. The AKT pathway is associated with increased cancer cell proliferation and survival [22]. Consequently, *CCNO* has other biological activities beyond cell cycle control. To investigate the unique roles of *CCNO*, we identified its interacting proteins using IP-MS. As is well known, the classic mode of action of cyclin is to bind to cyclin kinase in a dimeric form, activating downstream molecular signaling pathways through phosphorylation. A series of molecules found in the IP-MS,unexpectedly, we discovered that the CDK component involved in transcriptional regulation[24], *CDK13*, interacts with *CCNO*. *CDK13*, unlike cell cycle-associated CDKs, primarily regulates RNA transcription and gene expression by binding to cyclin K [23]. Our findings indicate that *CCNO* is a new

Reply: finished.

3. Table 1:
There is no symbol b in your Table 1, but you indicated its meaning in the Table footnote. Please

check.

III	14	25	

b, the median age at diagnosis in patients with LUAD was 60 years. Samples were

Reply: finished.
Changes: b have been deleted.

3. Figure 1:
Please check the meaning of "N" in Figure 1 legend. Should it be "Normal"?

adenocarcinoma; N, Node of lymph; T, Tumor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GEO, Gene Expression Omnibus; IHC, Immunohistochemistry.

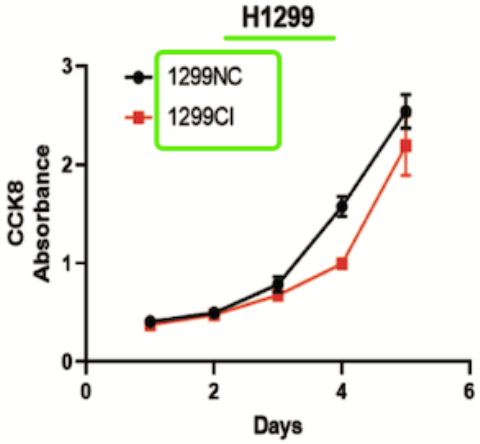
Reply: yes.
Changes: N, Normal.

4. Figure 2:
1) Please check the citation of Figure 2 in your main text. Figure 2D should not appears earlier than Figure 2C in your text.

compared to normal tumor cells (Figure 2A,2B). For tumor cells with knockdown *CCNO*, the lower *CCNO* cells have smaller clone number and colony size (Figure 2D). When the cell cycle was also examined, the tumor cells with knockdown *CCNO* were significantly blocked in the G0/G1 phase (Figure 2C). Regarding cell migration

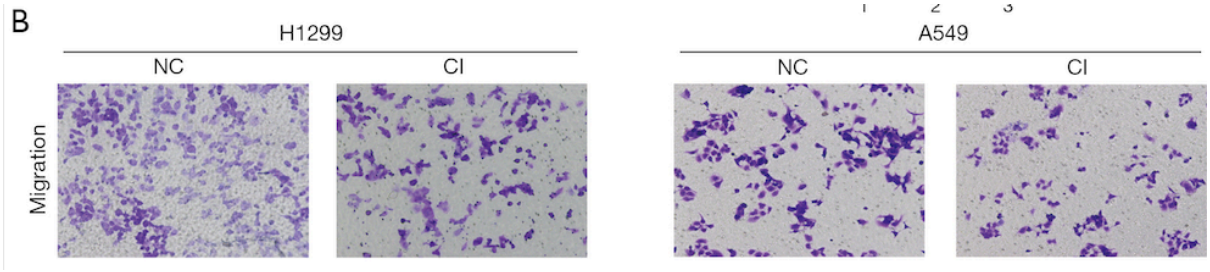
Reply: I'd like to adjust the paragraph.
Changes: When the cell cycle was also examined, the tumor cells with knockdown *CCNO* were significantly blocked in the G0/G1 phase (Figure 2C), which can be the potential causes of decelerated cell proliferation. For tumor cells with knockdown *CCNO*, the lower *CCNO* cells have smaller clone number and colony size (Figure 2D).

2) Figure 2A: Should the below "1299NC" and "1299CI be "H1299NC" and "H1299CI"? Please revise.



3) Figure 2C: Please check whether it's needed to add unit % in they-axis below.
4) Figure 2C: Please add the description in the x-axis.
5) Please indicate the full name of "G0/G1", "S", "G2M" in the legend.
6) Figure 2 is not clear. Please resubmit it in higher resolution.
Reply 4.2-4.6: figure 2 would be changed.

5. Figure 3:
Please indicate "Invasion" in Figure 3B. Which image is invasion cell maps?



Reply: invasion has been removed from legend.

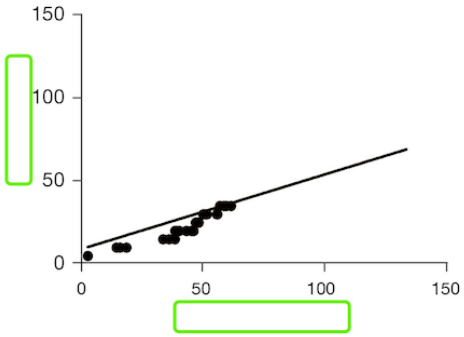
6. Figure 4:
Figure 4B legend and the main text don't match with Figure 4B. Please check. In Figure 4B, the *CCNO* is higher, but your legend and the main text is lower.

tumor injections. The weights (B) and volumes (C) are lower in xenograft tumors with *CCNO* knockdown than in xenograft tumors without *CCNO* knockdown. *, P<0.05.

tomors which were sourced from H1299-sh*CCNO* cells showed significantly less tumor growth and volume (Figure 4B,4C). The slowed growth volume and reduced

Reply: the statistics in figure4B are reversed. Changed.

7. Figure 5:
1) Figure 5H is cited in the main text, but no H image can be found in Figure 5. Please check.
2) Figure 5C-G legends and your main text all don't match with Figure 5C-G. Please check.
3) Please add the description of the x/y-axis in below image.



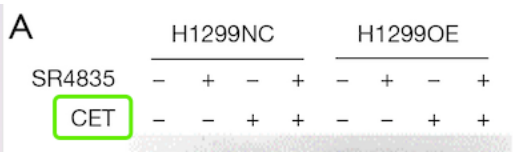
4) Please indicate the staining method and magnification for Figure 5F image in Figure 5 legend.

5) Please indicate the staining method and magnification for Figure 5G image in Figure 5 legend.

Reply: Figure 5 has been changed. Staining method and magnification I have declared.

8. Figure 6:

1) Figure 6A: Should the below word be "CE", as indicated in the legend?



NC, Negative control; OE, *CCNO* Overexpression; S, SR-4835; C, Cetuximab; P, Placebo; SR, SR-4835; CE, Cetuximab; *CCNO, Cyclin O*; *EGFR*, Epidermal growth

2) There is no symbol ** and **** in your Figure 6, but you indicated their meanings in the legend. Please check.

3) Please indicate the full name of "CI" in the legend.

Reply: finished.

9. Figure 7:

1) Figure 7C image can be found in Figure 7. Please check.

2) Please indicate the meaning of *, **, ****, ns in the legend.

3) There is Chinese brand name in the ruler. It's needed to remove it or hide it.

Reply: finished and figure 7 has be changed.