Peer Review File

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Reviewer A

In this manuscript, the authors demonstrated that overexpression of CD73 promotes the growth of human cervical cancer cell and transplanted tumor using in vitro and in vivo experiments. A part of the present findings may provide a novel insight into the mechanism by which CD73 promotes the tumor growth and metastatic property. However, the reviewer suggests several serious concerns as follows.

Major Comments

1. In Figure 1F, the proliferation rate of siHaNC cells appears to be higher than that of siHaCD73 cells, the results of which are inconsistent with the description in the Result section, Line 128-129.
2. The authors mentioned that it remains unclear how overexpression of CD73 leads to an increase in EGFR/AKT1 levels. Nevertheless, the authors should discuss more detail about the molecular mechanism by which overexpression of CD73 causes an increase in EGFR/AKT1 levels in HeLa and siHa cells by citing related previous papers in the Discussion section.
3. It is highly recommended to make a schematic diagram of the relationship among CD73, EGFR/AKT1, and cyclin dependent kinases such as CDK2, CDK3, and p21 as an additional Figure, which helps readers to understand this manuscript and the mechanism the authors proposed.
4. Throughout this manuscript, extensive editing of English language and style is absolutely required.

Reply 1：Thank you for your comment. The proliferation rate of siHaCD73 cells is higher than that of siHaNC cells. We have revised this in the revision.
Changes in the text: (Figure 1F)

Reply 2：Thank you for your comment. In this revision, we have add these description in the discussion section.
Changes in the text: (see Page 10, line 210-216)

Reply 3：Thank you for your suggestion. Accumulating data have showed that CD73 play important in cancer development. However, the function mechanism have not been fully understand. And moreover, the interaction mechanism between CD73 and other molecules, such as EGFR, AKT, p21, need more studies. Thus, to avoid misleading other readers, the sketch map could not be showed in this paper.

Reply 4：Thank you for your comments. In this revision, we have revised the English language carefully. If the language in the revision is still not sufficient quality, we can entrust

a professional organization to polish the language after the article were accepted.

Minor Comments

1. DMEM in Line 57, PCR in Line 61, and FITC in Line 75 seem to be necessary to be spelled out when appeared for the first time.
2. The section of Statistical analysis should be written more carefully. For example, does "Data comparisons" in Line 119-120 mean "Comparison between two groups"?
3. In Line 97, (PI) seems to be a typo.

Reply 1：We have modified our text as advised.
Changes in the text: (see Page 3, line 65, Page4 line 70, Page4 line 85)

Reply 2：We have modified our text as advised.
Changes in the text: (see Page 7, line 139)

Reply 3: We have modified our text as advised.
Changes in the text: (see Page 6, line 111)

Reviewer B

Strengths – a mechanism that can be relevant in finding a new target for cervical cancer treatment.
Limitations - addressed by the authors at the end of the discussion.
Grammar should be revised throughout the entirety of the text.
The importance of CD73 in normal physiology and in cancer is lacking, more information on the topic could be interesting to speculate possible mechanisms by which the EGFR/AKT1 pathway is activated. Also, little is discussed on the importance of the EGFR/AKT1 pathway in cancer in general and, specifically in cervical cancer, although EGFR is expressed in about 80% of cervical carcinomas and correlates with disease progression. Its activation may trigger several signaling pathways, including AKT, promoting tumor progression. This information would highlight the importance of the findings presented by the authors and should be included.

Also, which statistical analysis was used in each data set should be included in each caption.
Major: in lines 128-129 is mentioned that the proliferation of SiHa cells overexpressing CD73 is higher than in control cells, however, in figure 1F appears that the proliferation of SiHanc is higher. This should be immediately addressed, due to the direct contrast between the text and the data presented, and the importance of this data to the study.

Response:

Reply to Q1: Thank you for your comments. In this revision, we have revised the English language carefully.

Reply to Q2: We added these data in the discussion section.
Changes in the text: (see Page 9, line 186-189, Page 9-10, line193-200)

Reply to Q3: All data comparisons between two groups in this study were conducted by using the Student's t-test, which we have listed in the Statistical Analysis Section.
Changes in the text: (see Page 7, line 139-140,)

Reply to Q4: The proliferation rate of siHaCD73 cells is higher than that of siHaNC cells, We have revised this in the revision.
Changes in the text: (Figure 1F)

Reviewer C

In this manuscript, Liu et al. investigate the role of the EGFR/AKT pathway in cervical cancer. Whilst an advance on their previous study, some essential revisions are required before publication. Please see my specific comments below.

- In the introduction/discussion, the authors should mention HPV as this is a critical factor in the development of the vast majority of cervical cancers, particularly as HPV actives both EGFR signalling (e.g. Zhang et al., Oncogene, 2005; Spangle et al., Plos Pathog, 2013; Wasson et al., Oncotarget, 2017; Morgan et al., Cell Death Differ, 2021) and AKT signalling (e.g Menges et al., Cancer Res, 2006; Morgan et al., Plos Pathog, 2019). These studies should be referenced and discussed as they will add essential details for the reader
Reply1：Thank you for your comment. In this revision, we have add these description in the discussion section.
Changes in the text: (see Page 9-10, line193-200)

- Line 52 - please remove sentence, it is unnecessary
Reply2：Thank you for your comment. This sentence is a magazine format requirement

- Fig 1 - please increase size of images in 1H
Reply3：We have modified our text as advised
Changes in the text: (figure1H)

- Fig 2A and B - western blot data for both EGFR and AKT should be shown in HeLa and SiHa expressing CD73
Reply 4: Thank you for your comment. The increased EGFR and AKT in cervical cancer cell

with CD73 overexpression have been demonstrated in our published paper (PMID:28202050). Thus, in this paper, we only detected the mRNA expression for EGFR and AKT in these cells.

- Fig 2D and E - western blot data for both EGFR and AKT after siRNA mediated knock down MUST be shown

Reply5：We added western blot data for both EGFR and AKT after siRNA mediated knock down

Changes in the text: (Figure 2F)

- Fig 3A and B - please quantify this data from at least 3 replicates

Reply6：Thank you for your comment. We added the cycle data of knockdown of EGFR and AKT1 in Hela^{CD73}/Siha^{CD73}, and performed statistical analysis

Changes in the text: (see Figure 3A and B)

- Fig 3C and D - western blot data from the genes identified from mRNA analysis would be informative (CDK2, CDK3 and p21). Also, please refer to the gene name, NOT the protein name (e, g, p21 gene name is CDKN1A)

Reply7：Thank you for your comment. Due to the impact of COVID-19, we can't get the antibodies in time. Thus, in this revision, the WB results of CDK2, CDK3 and CDKN1A could not be added. Even so, the mRNA expression results of CDK2, CDK3 and CDKN1A still supported our conclusion in this paper. The function mechanism between EGFR/AKT and these molecules (CDK2, CDK3 and CDKN1A) would be further researched in our next study.

- Fig 4 - the apoptosis data is not convincing at all. The data from EGFR/AKT knockdown appears to be much higher than 3-5% of the total cells (in particular in SiHa, EGFR KD cells). How was the apoptosis rate defined? This needs to be clarified.

Reply8：Thank you for your comment. We statistically analyzed the apoptotic cells double-stained with annexin V-FITC and PI. In this revision, We have added a description of apoptosis statistics

Changes in the text: (see Page 6, line114-115)