Peer Review File

Reviewer Comments

Major comments -

1) Methods section is lacking. What MOI of lentivirus was used for infection? No secondary antibody information for IHC. No details on antibodies for western blotting.

Reply 1: Thank you for your comments.

The MOI of 786-O and 769-P cells was 5, and the MOI of ACHN and Caki-1 cells was 10. Staining for CD73 was underwent using a rabbit monoclonal anti-CD73 antibody (1:100, ab133582, Abcam) and goat anti-rabbit secondary antibody (BD5100, Bioworld). The antibodies for western blotting were as follows: CD73 (ab133582, Abcam), p-JNK (#4668, Cell Signaling technology), EGFR (ab52894, Abcam) and GAPDH (bs-0755R, Bioss).

Changes in the text: We have modified our text as advised. (see Page 4, line 119-120 and Page 5, line 133-144)

2) Figure legends are not detailed enough. Please define N and T in legend (I know it is normal vs. tumor but might not be obvious to some readers). Figure 1 legend needs more details, are these 8 random tissues for western blot? Where did Fig 1C tissue sections come from?

Reply: Thank you for your comments.

Figure 1. CD73 is upregulated in ccRCC, and high expression of CD73 is correlated with poor prognosis. (A) Western blot of 8 fresh pairs of random tissues showed CD73 was upregulated in ccRCC tissues compared with that in the matched normal tissues. T, tumor tissues; N, matched normal tissues. (B) CD73 was overexpressed in RCC samples from the TCGA and three GEO datasets. (C) Representative images of low expression (a, b) and high expression of CD73 (c, d) from the paraffin specimens of the included 72 patients. (D) Kaplan-Meier analysis showed patients with higher expression of CD73 had a worse overall survival.

Changes in the text: We have modified our text as advised. (see Page 14, line 397-403)

For Figure 2, are the errors bars standard deviation or standard error. How many blots were used to generate graphs? Can graphs be made larger so they are easier to read?

Reply: Thank you for your comments.

The errors bars are standard deviation. All experiments were performed in triplicate. The graphs were appropriately enlarged.

Changes in the text: We have modified our text as advised. (see Page 14, line 405-406)

Figure 3, are error bars standard deviation? How many experiments were performed per analysis? Missing scale bar in images. Figure 3 legends need more details, how long was wound healing and transwell assay performed for? Xenograph of what cell line for in vivo assay? Phospho JNK blot showing decrease of p JNK is not convincing, could you provide analysis of the multiple experiments showing change in phosphorylation?

Reply: Thank you for your comments.

The errors bars are standard deviation. All experiments were performed in triplicate. Wounds healing assay showed that suppression of CD73 inhibited cell migration as observed after incubation for 24 hours. Transwell assay showed that suppression of CD73 inhibited cell migration as observed after incubation for 24-36 hours. Xenograft assay showed that tumors treated with knockdown of CD73 in 769-P cells grew more slowly.

Changes in the text: We have modified our text as advised. (see Page 14, line 408-415)

Figure 4, same comments as above.

Reply: Thank you for your comments.

The errors bars are standard deviation. All experiments were performed in triplicate. Wounds healing assay showed that overexpression of CD73 enhanced cell migration as observed after incubation for 24 hours. (D) Transwell assay showed that overexpression of CD73 enhanced cell migration. as observed after incubation for 24-36 hours. (E) Xenograft assay showed that tumors treated with overexpression of CD73 in ACHN cells grew more fastly.

Changes in the text: We have modified our text as advised. (see Page 14-15, line 417-424)

Figure 5, same comments as above.

Reply: Thank you for your comments.

The errors bars are standard deviation. All experiments were performed in triplicate. Wound healing assay showed that APCP inhibited the effect of the CD73 overexpression on cell migration as observed after incubation for 24 hours.

Changes in the text: We have modified our text as advised. (see Page 15, line 426-430)

3) Did the authors try CD73 knockdown experiments in cell lines with lower CD73 to see if further reduction of CD73 showed same phenotype as expected? Likewise with CD73 overexpression. It would have been interesting to also have performed either orthotopic injection of kidney cancer cells in mice and/or performed experimental metastasis assay to show metastatic formation of C73 silenced or overexpressed tumor cells show expected phenotype.

Reply: Thanks for your suggestions. It's really much more convince to undergo CD73 knockdown/overexpression experiments in cell lines with lower/higher CD73 and perform orthotopic injection of kidney cancer cells in mice. We will undergo these experiments in our further studies. Thanks again.

4) More discussion should be included on what the potential mechanism behind CD73 regulation of ccRCC progression. Authors suggest there are non-enzymatic and enzymatic activities but only provide minor discussion on these activities.

Reply: Thanks for your suggestions. We have discussed the potential mechanism behind CD73 regulation of ccRCC progression in more detail according to your suggestion.

Changes in the text: We have modified our text as advised. (see Page 10, line 274-277).

5) Have you tried CD73 antagonist in vivo to see if it can inhibit tumor growth?

Reply: Thanks for your suggestions. It's really much more convince to use CD73 antagonist in

vivo to see if it can inhibit tumor growth. We will undergo these experiments in our further studies. Thanks again.

Minor comments -

1) Grammatical errors throughout the document detract from the manuscript

Reply: Thank you for your comments. We have revised the manuscript carefully, and the main text has been checked by a native English-speaking expert.

2) Discussion repeats itself in some sections (paragraph starting with line 224 and second paragraph starting with line 249)

Reply: Thank you for your comments. Your suggestion really means a lot to us. We have revised the discussion section according to your suggestion.

Changes in the text: We have modified our text as advised. (see Page 10, line 226-229).

3) Typos in table 1 (P valuea*). Typo in Figure 4 (vetor*)

Reply: Thank you for pointing out such mistake we have made. We have revised those typos.

Changes in the text: We have modified our text as advised. (see Table 1 and Figure 4).