Author’s response to reviews

Title: Differential CARM1 Expression in Prostate and Colorectal Cancers

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Response to Editor/Reviewers

Referee #1 (Comments to the Author):

Minor essential point
1. “On fig2A-B CARM1 expression assessed by RT-PCR and immunoblotting in various cell lines does not always fit and these discrepancies are not mentioned and/or discussed. This aspect should be added and discussed in the paper.” We revised our manuscript to accommodate reviewer’s rightful comment in discussion section. Briefly, our expression data showed high expression of CARM1 in only PC3 and DU145 cells. While these cells are described as androgen-independent prostate cancer cells, they are generally considered as ‘non-prostate-like cancer cells’ mainly due the deficiency of androgen receptors. Therefore, conclusion from our patient samples implying that CARM1 is not overexpressed in hormone refractory prostate cancers well correlate with this phenomenon.

2. “English language should be checked, there are typing mistakes left.” English editing is newly done by professional editor.

3. “Data showing the lack of co-activation of P300 in p53-mediated transcription activation should be shown in the main text or at least in the suppl data and not as data not shown.” As reviewer suggested, new data including p300 cotransfection was added into Figure 5C.

Referee #2(Comments to the Author):

Major compulsory revisions:
1. “~ And, be recommended to see the interaction of CARM1 to each promoter region. Chromatin immunoprecipitation (ChIP) to each cell-lines might be needed.” As suggested by reviewer, ChIP assay was done using promoters of
p21 and cyclin D1 in caco2 colon cancer cells. Figures are newly arranged to accommodate new ChIP data in Figure 6.

2. “The localization of CARM1 in each cells should be examined to compare the function of transcriptional activity.” While we appreciate reviewer’s comment, we are not sure whether this type of additional experiment will add any valuable information to strengthen our result. We conclude that CARM1 is overexpressed in colorectal cancer cells but not in prostate cancer cells. We showed this phenomenon by Western blot (Figures 2B and 3C) in addition to tumor samples. As we described, high expression of CARM1 in some prostate cancer cells (i.e. PC3 and DU145) may come from non-prostate-like phenotype shown by this type of androgen receptor-deficient cells. While it may be interesting to show cellular localization of CARM1 in various cell types, it is generally accepted that coactivators are usually evenly distributed throughout the cell unless there is trafficking machinery involved or postmodification of proteins. Taken together, we believe that reviewer’s point will be addressed in our future study when we will be able to perform more extensive promoter study.

Minor compulsory revisions:
1. “Lusiferase assays with knocking out system and dominant negative CARM1 expression will be helpful to discuss the feature of CARM1.” As reviewer pinpointed, basically our reporter transcription assay was the repetition of already well established phenomenon as an attempt to find presumable mechanism on CARM1 in colorectal tumorigenesis. Therefore, we feel that addition of data to show specificity of CARM1 is not much helpful to draw our conclusion, considering time and efforts. I’d like to note that our attempt to develop CARM1 SiRNA was failed and showed no specific targeting. To be honest, this is not inexpensive experiment to repeat.

2. “With the expression of mRNA, that correlate each reporter plasmid will make clear the relationship between tissue micro array and lusiferase assays.” We feel sorry that we do not understand reviewer’s point. If reviewer suggests performing RNA expression in both tissue microarray and luciferase sets, that type of assay is not currently feasible.

Discretionary revisions:
“Fig.5 D: Transcriptional activity of TCF4RE seems to be increased by CARM1. Are there any events of cell cycle change at CARM1 over expression?” Opposed to reviewer’s view, CARM1 did not increase TCF4RE response on top of p300 activity (refer Fig. 5E). We do not know if there is any cell cycle change.

-End of Rebuttal-