Author's response to reviews

Title: Differential CARM1 Expression in Prostate and Colorectal Cancers

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Author's response to reviews: see over
April 28, 2010

Dear Editor,

RE: Second resubmission of MS: 2111478509325193 - Differential CARM1 Expression in Prostate and Colorectal Cancers

Thank you for favorably considering our manuscript for the publication in BMC Cancer.

As described below, appropriate revisions or answers are made on opinion of reviewers. We hope we made enough revision to accommodate reviewer’s concern for this manuscript to be published in BMC Cancer in the near future. Please let me know if you have further suggestion(s). Again we appreciate your help getting our manuscript reviewed.

Sincerely,

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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.” As reviewer suggested, revision has been made in discussion section as follows (page 14 lines 15-22):

In cultured cancer cells, mRNA and protein level of CARM1 were not always correlated, especially in 231 breast cancer cells and CWR22RV PCa cells. This discrepancy are not clearly understandable. Ohkura et al have demonstrated that there are at least one other isoform of CARM1 existed in endogenous cell level by alternative splicing [1]. This isoform may have contributed to increased CARM1 protein expression compared to the level of RNA. It is also noteworthy that, in addition to its transcriptional coactivator role, CARM1 also regulates target gene expression by modulating protein stability, including p/CIP [2], AP-1 [3], and NF-κB [4].

Minor compulsory revisions:

1. "~ RNA silencing system using shRNA plasmid might be another way to knock out CARM1 expression” We have consistently pursued to identify CARM1-specific SiRNA in our hands, which ultimately all failed. Instead, we have employed CARM1 dominant-negative mutants shown by Higashimoto et al. Single point mutation at S229E eliminates enzymatic activity of CARM1. This mutant showed suppressive effect on endogenous CARM1 activity in TNFα-stimulated NF-κB response as shown in Fig. 5E. Appropriate revisions are made in results and M&M sections (Page 6 lines 6-8 & page 12 lines 20-23).


2. “Authors need to show some biological evidences of p53 and NF-κB mediated cancer progression.” Biological evidence of these pathways in cancer development are discussed in the section as follows (page 16 lines 6-14):

   Both NF-κB and p53 pathways are key mediators of genes involved in the control of the cellular proliferation and apoptosis [5-6]. Antiapoptotic genes that are directly activated by NF-κB include c-IAP1, c-IAP2, and IXAP, TNF receptor–associated factors, the Bcl-2 homologue A1/Bfl-1, and IEX-IL [7]. NF-κB directly induces expression of A1/Bf1-1 by binding to specific sites in its promoter [8]. NF-κB also acts in the control of the cell cycle. NF-κB activates the expression of cyclin D1, a positive regulator of G1-to-S-phase progression, by direct binding to multiple sites in its promoter [9]. Thus, the apoptotic regulation by either NF-κB or p53 involves the regulation of multiple genes involved in different aspects of growth control.


-End of Rebuttal-