Author's response to reviews

Title: miR-17-5p targets the p300/CBP-associated factor and modulates androgen receptor transcriptional activity in cultured prostate cancer cells

Authors:

Ai-Yu Gong (aiyugong@creighton.edu)
Alex N Eischeid (alex-eischeid@uiowa.edu)
Jing Xiao (jxiao@bcm.edu)
Jian Zhao (zj804@163.com)
Dongqing Chen (dongqingchen@creighton.edu)
Zhao-Yi Wang (zhaoyiwang@creighton.edu)
Charles YF Young (youngc@mayo.edu)
Xian-Ming Chen (xianmingchen@creighton.edu)

Version: 2 Date: 30 June 2012

Author's response to reviews: see over
June 28, 2012

Xian-Ming Chen, M.D.
Medical Microbiol & Immunol
Creighton University
Omaha, NE 68178
Phone: (402)-280-3750
Fax: (402)-280-1875

The Editor
BMC Cancer

Dear Editor:

Thank you for your letter of May 1, 2012, regarding our manuscript (1984867920629801), entitled “The p300/CBP-associated factor modulates androgen receptor-regulated transcriptional activity and cellular growth in cultured prostate cancer cells.” We were pleased that the reviewers found that our findings “are important to those with closely related research interests.” We very much valued your suggestions and those of the reviewers, and appreciate the opportunity to provide you with a revised manuscript for consideration for publication in BMC Cancer.

We believe that we have adequately responded to all the concerns that were raised. Specifically, per suggestions from the reviewers, we have significantly modified the manuscript including: i) revision of the title to actually reflect its content; ii) addition of information about the methodologies and resources; iii) deletion of the PEITC data to focus on the targeting of PCAF by miR-17-5p; iv) addition of new data confirming our previous findings on PCAF mRNA level in the cells; and v) description of the ethical requirement for the use of the samples in research.

For ease of review, the revised portions in the manuscript are highlighted in blue. Enclosed please also find a copy of point-by-point responses to the reviewers’ comments. We believe that the manuscript is considerably stronger thanks to the quality of the review. We hope you agree.

Sincerely,

Xian-Ming Chen, M.D.
Professor
Dept. of Medical Microbiology and Immunology
Creighton University Medical Center
2500 California Plaza
Omaha, NE 68178
E-mail: xianmingchen@creighton.edu
We thank all the reviewers for your positive comments on our manuscript. We appreciate your thoughtful review and have attempted to satisfactorily respond to your concerns and suggestions.

Responses to comments from Reviewer #1

1). The protein level of PCAF in Fig 1A seems not in parallel with mRNA level in Fig 1B. Is there any possible explanation? No method of statistical analysis was given, and it is not clear how many independent experiments were performed. In addition, it is hard to believe there was a statistically significant difference of mRNA level, in particular between PrEC and LNCaP (Fig 1B). We agree with you that the protein level of PCAF in Fig 1A was not in parallel with the mRNA level in Fig 1B. Specifically, PCAF mRNA was detected in RWPE1 and PrEC cells, but PCAF protein was barely detectable in those cells. This inconsistency stimulated us to test a potential role of miRNA-mediated post-transcriptional suppression in PCAF expression in prostate epithelial cells. Indeed, targeting of 3'UTR of PCAF by miR-17-5p caused translational suppression and degradation of PCAF mRNA. Importantly, a higher level of miR-17-5p was detected in RWPE1 and PrEC cells than that found in several prostate epithelial cancer cell lines. This miR-17-mediated post-transcriptional suppression of PCAF may partially explain the above inconsistency between PCAF mRNA and protein levels. Nevertheless, other post-transcriptional mechanisms may also be involved, including PCAF protein degradation and involvement of other miRNAs. We have revised our manuscript and included discussion on those possibilities (page 16, lines 23–25; and page 17, lines 1–2). We have also added methods of statistical analysis and included information on the number of independent experiments performed for all the figures (page, 11, lines 1–3; page 22, lines 23–24; page 24, line 4; and page 25, lines 4 and 18). Moreover, we have repeated the real-time PCR analysis of PCAF mRNA levels in these cell lines and our new data confirmed the statistically significant difference of PCAF mRNA level between these cell lines. We have included our new data in the revised figures (new Figure 1B).

2). It seems that both pCX-PCAF and PCAF siRNA worked very well (Fig 2A), however only minimal decrease or increase of PSA mRNA were seen in Fig 2B. We agree with you that, although statically significant, only about 50% inhibition by siRNA and two-fold increase by pCX-PCAF on DHT-induced PSA expression were detected in LNCaP cells as shown in Fig 2B. The minimal effects of PCAF siRNA or pCX-PCAF transfection on DHT-induced PSA expression detected in LNCaP cells may be due to the basal high level of PCAF in the cells. We have modified the text to reflect this issue (page 12, lines 4–6).

3). Data from Fig 2E showed that pCX-PCAF itself stimulated LNCaP cell growth in the absence of DHT. This result suggests that stimulation of prostate cancer cell growth by PCAF is not necessarily via AR. We want to thank you for pointing out this important point. Although we cannot exclude the possibility that potential stimuli from the culture medium may activate AR signaling in the absence of DHT, we agree with you that overexpression of pCX-PCAF may stimulate prostate cancer cell growth through AR-independent mechanisms. We have included discussion on this possibility in the revised manuscript (page 15, lines 19–22).

4). There is a considerable difference of protein level of PCAF in LNCaP between Fig 1A and Fig 3D, is there any possible explanation? A lower amount of total protein was loaded for the Western blot analysis in Fig 3D, indicated by the weaker bands for actin in Fig 3D. To keep these two sets of data consistent with each other, we have repeated the
experiments using the same amount of total protein as in Fig 1A and have included a new blot gel in the revised manuscript (new Fig. 3D).

5). **No data support the statement on page 9, beginning at line 16 “Cells transfected with the empty vector or treated with the non-specific scrambled siRNA showed no changes in DHT-induced PSA luciferase activity.”** We apologize for our ambiguous description in this paragraph. We have data demonstrating that: 1) cells transfected with the empty vector or treated with the non-specific scrambled siRNA showed no changes in DHT-induced PSA luciferase activity; and 2) pCX-PCAF and PCAF siRNA displayed a similar effect on DHT-induced PSA expression in C4-2B cells as in LNCaP cells. In the previous version of the manuscript, we wanted to describe this as “data not shown” for both statements. Per your suggestion, we have modified the text to make it clearer (page 12, lines 12–16).

6). **The manuscript requires editing.** We have carefully checked the text and corrected these spelling and grammatical errors.

**Responses to comments from Reviewer #2**

1). **The title is inadequate and gives no indication of what the paper is actually about.** Per your suggestion, we have revised the title to “miR-17-5p targets the p300/CBP-associated factor and modulates androgen receptor transcriptional activity in cultured prostate cancer cells”

2). **The manuscript is generally very poorly referenced. This is a very busy field, indeed much of this work has already been published elsewhere, and this study really needs to be placed into the context of the numerous studies and not just individual selected publications.** We agree with you and have revised the Background section to include general references in the field (page 5, lines 5–7, 10–25; page 6, lines 1–4).

3). **The studies are generally performed to a good standard although the materials and methods lack sufficient detail in key areas; what was the vehicle for DHT, what concentrations were used for the antibodies, clarify what CCS is and where purchased, how much DNA was added for the PCAF 3'UTR assay, how long were cells grown prior to the MTS assay, how was statistical significance calculated?** This is an important issue and we thank you for your suggestions. We have carefully checked the manuscript and included sufficient details for the materials and methods, including vehicle for DHT, antibody concentrations, DNA amount for 3'UTR assay, and statistical analysis, etc. (page 6, lines 21–22; page 7, lines 3–4, 7–18; page 8, lines 23–24; page 10, lines 2–4, 11 and 17; page 11, lines 1–3).

4). **There was no information at all on how the immunofluorescence and IHC were performed.** A detailed description of immunofluorescence and IHC is included in the revised manuscript (page 7, lines 7–18).

5). **There are no controls for the fluorescence or IHC, particularly for antibody specificity and it not sufficient to say PCAF is up-regulated in prostate cancer tissue without scoring the entire TMA and presenting the data.** We want to thank you for your suggestion to include controls for the fluorescence and IHC. In the revised manuscript, we have included images using the control non-specific antibodies, demonstrating the specificity for these antibodies used for the fluorescence and IHC. We have also included images for entire tissue samples (page 7, lines 7–18).

6). **How did the authors control for the effects of endogenous miR-17-5 and PCAF and their effect on their constructs? It is far from clear that PCAF is regulated by miR17-5 and not that PCAF is required for miR17-5 function.** From a technical point of view, it is very difficult to finely control the effects of endogenous miR-17-5p and PCAF. To address
this important issue, we have used both the anti-senses (anti-miR to miR-17-5 and siRNA to PCAF) and forced expression (precursor to miR-17-5 and pCX-PCAF) in our study. Integrated interpretation of data from these experiments should help to reveal the effects of endogenous miR-17-5 and PCAF. We agree with you that it is far from clear that PCAF is regulated by miR17-5 and not that PCAF is required for miR17-5 function. Data from our luciferase reporter analysis with the PCAF 3'UTR demonstrate targeting of PCAF by miR-17-5p. However, each miRNA may have multiple targets, so miR-17-5p may also regulate other targets to modulate AR signaling in prostate cells. Therefore, PCAF may not be required for all the functions for miR-17-5p. We have included discussion on this possibility in the revised manuscript (page 16, lines 23–25; and page 17, lines 1–2).

7). It is not clear why cells were treated with PEITC and what this has to do with the story being told here. Most of these effects are already published and the only novel effect is on the microRNA and the authors could focus on this instead of all of the previously published data. On reading this manuscript appears to be a series of well conducted but random experiments that are only connected by PCAF. There is no logical progression from miR-17-5p to PEITC – what is the link? Why look at PEITC at all? The initial studies on PCAF and AR in LNCaP cells have all been completed before in larger and more in depth studies which are not mentioned in this manuscript. The miR-17-5 data is conducted well and is more novel and I suggest the authors might be better to focus on this data. We want to thank you for your constructive comments and suggestions. To address your above concerns in general, we have significantly modified the manuscript including: i) revision of the title to reflect its content; ii) modification of the Background to include additional background information on PCAF and AR signaling in prostate cancer cells; and iii) deletion of PEITC data (page 3, lines 3–25; page 4, lines 2–4; page 5, lines 6–25; page 6, lines 1–9; page 14, lines 22–25; and page 15, lines 1–5). Overall, the manuscript reads much better; thank you again for your suggestions.