Author's response to reviews

Title: Stable Alterations of CD44 Isoform Expression in Prostate Cancer Cells Decrease Invasion and Growth and Alter Ligand Binding and Chemosensitivity

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Author's response to reviews: see over
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Dr. Sabina Alam  
Senior Scientific Editor,  
*BMC Cancer*  

Dear Dr. Alam:

We are most grateful for the two reviewers’ comments on our manuscript, “Stable alterations of CD44 isoform expression in prostate cancer cells decrease invasion and growth in vitro and alter ligand binding and chemotherapy sensitivity.” We now resubmit this work for *BMC Cancer*.

**Reviewer 1 (Subramaniam)**

1. In response to the comment, all Figure Legends have been revised to summarize rather than restating the results. Legends were shortened. Some of the extra verbiage in the captions was transferred to the text under Results.

2. The need to avoid “Tx” was mentioned. In Figures 3, 4, and 5, “No Tx” has been replaced by “Control.”

3. It was suggested that the same shading pattern be used for Figures 4, 5, and 6, that is used for Figure 3. Figures 3 and 6 already have identical shading patterns. Thus, the shading for Figures 4 and 5 has been modified.

4. It is asked, what is the rationale for choosing PC-3 cells? In fact, we used PC-3M cells. We used PC-3M because of its very high CD44v7-10 expression, as we documented in Robbins et al, *BMC Cancer* 2008. Thus, cells having a high baseline CD44v7-10 should show the most dramatic reductions, either through RNAi, or by counteracting the expression by re-expressing CD44s. It is asked, since cells are AR-, could androgen ablation play a role in the results? As long as the cells with altered CD44 are being compared to luciferase-only cells, all grown in the absence of androgen (other than trace amounts in fetal bovine serum), the absence of androgen is controlled for. Whether alteration of CD44 expression affects AR is a topic for another study.
We do not know whether the high CD44v7-10, a result of aberrant splicing, is due to androgen insensitivity (not really androgen *ablation*). However, we have consistently found CD44v7-10 to be higher in PC-3 and its derivatives than in androgen-sensitive LNCaP (Iczkowski et al., “Paracrine calcitonin…,” 2005).

5. We thank the reviewer for pointing out that the effect of CD44s is opposite in colon cancer and prostate cancer cells. This misstatement has been corrected (bottom of page 11). I also rearranged the order of sentences for clarity. Interestingly, though, Harada et al. documented an anti-growth effect of CD44s in colon cancer only *in vivo*, but not *in vitro*.

6. For Figure 6, the need to indicate p-values for Docetaxel sensitivity was cited. We now have normalized the values at dose=0 to each other, since the ODs were slightly different (range 1.4 to 1.7). This is explained (pg. 8). This enabled us to do a t-test and indicate with stars which values are significantly (p<0.05) different from control cells for each Docetaxel dose (2.5, 5.0, …).

**Reviewer 2 (Culig)**

1. The purpose of the study and how it advances previous work needed explanation. The Introduction has been revised with this issue in mind.

2. Two very recent, 2009 papers by Palapattu et al., and Simon et al. were mentioned that deal with high CD44 in neuroendocrine prostate cancer. In these studies, CD44 is said to be mostly absent in non-neuroendocrine prostate cancer. These references [18,19] have been added to the Discussion (pg. 12 top). These studies used the IM7 rat anti-mouse monoclonal antibody against HCAM/Pgp, and the DF1485 mouse monoclonal antibody against HCAM/Pgp. Both of these antibodies are raised against lymphocyte membrane and detect a 90-kD protein consistent with CD44s. When we used the DF1485, it was to detect CD44s [14](Robbins EW et al., BMC
Cancer. 2008; 8: 260). These antibodies do not detect the epithelial CD44v8-10 or the v7-10 of non-neuroendocrine prostate cancer. Thus, their finding of nearly absent staining in prostate cancer other than neuroendocrine cancer is consistent with our result of loss of CD44s and some other variants in cancer, but strong CD44v7-10.

3. and 4. The Materials and Methods section has been revised to include numbers of animals used, and the details of approval by the Institutional Animal Care and Use Committee.

5. It is stated that the RNAi experiments lack controls. Indeed we did not perform a separate set of experiments with cells transfected by sense RNAi as a control. We admit, in the Discussion, (middle of pg. 12) that this is a limitation of the study, precluding ruling out some non-specific effects of RNAi. However, the CD44-luciferase control cells are virally infected, ruling out any nonspecific effect of viral infection per se on functional assays or in vivo growth.

6. The need to discuss the mechanism of Docetaxel sensitivity is mentioned. References 24 and 25 on were installed (pg. 12), showing that CD44 variant expression in other cells is anti-apoptotic and reference 17 is cited for pro-growth effect of CD44 variant. (Although as mentioned in the previous item, the specificity of our RNAi was not thoroughly assessed).

7. It is suggested we reduce the focus of the Discussion section on merlin. We have responded by considerably shortening the paragraphs in the Discussion discussing merlin. Some of the sentences were repetitive. The Discussion was made more balanced, as recommended, by focusing on previous CD44 studies in prostate cancer, as related to growth and other in vitro experiments, including the addition of the two neuroendocrine papers mentioned in item 2 above.

Sincerely,

Kenneth A. Iczkowski, M.D.

Kenneth A. Iczkowski, MD