Author's response to reviews

Title: Further Evidence for Increased Macrophage Migration Inhibitory Factor Expression in Prostate Cancer

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Author's response to reviews: see over
To Whom It May Concern:

Enclosed is our revised manuscript “Further Evidence for Increased Macrophage Migration Inhibitory Factor Expression in Prostate Cancer” for review by the editorial board of BMC Cancer. We thank the reviewers for their insightful comments. Below is listed a point by point response to each of the reviewers:

Reviewer comments: Dr. Bacher

**Minor Essential Revisions**

1. We discussed the possible conformation of physiologically active in vivo MIF: Discussion pg. 18, 2nd paragraph

Reviewer comments: Dr. Mitchell

**Major Compulsory Revisions**

1. We have completely revised Figure 3 adding additional data and detailed the methods.
   a. Methods in abstract now include reference to native, denaturing and reducing polyacrylamide gels.
   b. Methods section details native, denaturing and reducing polyacrylamide gels, MIF forms in human serum, pg 7.
   c. Results section, MIF forms in human serum, pg 13. Serum proteins under native conditions show a high molecular weight band (150 – 500 kDa, Fig 3 A). Under denaturing conditions this resolves into a single band of 180 kDa, Fig 3B. This complex is resolved into the MIF monomer under reducing conditions, Fig 3 B, Results section pg 14 paragraph 2.
   d. These banding patterns are also seen with monoclonal antibody III.D.9. This antibody wasn’t used in these Western blots since the comparison was to the MIF detected by ELISA and Western blot using the R&D Systems antibody.

2. We have changed the first paragraph of the discussion section to: Recently attention has been focused on the possibility that epithelial cell injury as a result of chronic inflammation plays an important role in prostate carcinogenesis. Thus, we proposed that MIF, a proinflammatory cytokine constitutively expressed by the prostatic epithelium and upregulated in prostate cancer, plays an important role in this disease entity.
Minor Essential Revisions

1. The total magnification of the figure 4 is 400X, the ocular was 10 X magnification. The figure legend was revised to list 400X as the total magnification.

2. We discussed the studies which document that immobilized MIF does not bind BSA at concentrations to 2.5 mg/ml.
   a. The abstract contains references in results and conclusion sections that state that the ELISA diluent reagents that included BSA significantly reduced serum MIF detection.
   b. We discuss the results of the albumin subfraction study in greater detail, Discussion, pg. 17. We note that in the ELISA the BSA concentration is four fold greater than in the immobilized MIF binding study. In addition we note that in our study the MIF-BSA interaction would occur in solution. We conclude that BSA interference is a likely explanation for the discrepant findings as there were no other major differences in the serum diluent/blocking agent used including pH and ionic strength.

We look forward to the review of our revised manuscript. If you have any questions, please do not hesitate to contact us.

Sincerely,

Katherine L. Meyer-Siegler, Ph.D.        Pedro L. Vera, Ph.D.