Reviewer's report

**Title:** Inhibition of Macrophage Migration Inhibitory Factor Decreases Proliferation and Cytokine Expression in Bladder Cancer Cells.

**Version:** 1  **Date:** 17 May 2004

**Reviewer:** Marcia R. Saban

**Reviewer's report:**

General

This is a very interesting manuscript dealing with an extremely relevant disorder. The investigators indicate that MIF is localized within human bladder urothelial cells and several treatments that alter MIF amounts have a potential for treating bladder cancer.

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**Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)**

1-What would be the explanation for this cell line (HT-1376) to release all 65 proteins in the cultured medium? Is there any support in the literature indicating that this cell line produces: NGF, other neurotrophins, leptin, and SCF?

2-All examined treatments significantly altered the secretion of a wide range of cytokines (page 17). If this is the case, what is the fundamental physiological basis for MIF action? Can the ERK1/2 pathway be responsible for alteration such array of alterations?

3-On Page 12, Anti-MIF antibody effect. I am not sure why anti-MIF antibody did not abolished detection of MIF by ELISA and western. In other words, is the complex MIF-antibody still detectable by ELISA and Western? Could the authors really conclude that MIF is not being released or detected?

4- Page 13 and Figures 3C and 3D. What would be the mechanism by which MIF antibody reduces mRNA levels (Figure 3D). It is a rather unusual finding that an antibody against a protein or peptide would reduce both the protein and its mRNA levels.

In addition to MIF mRNA, TNF-alpha mRNA was also decreased. Have the author used a control mRNA?

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**Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)**

1- Abstract. “MIF protein is secreted…..” Please, consider revising: “Human bladder cancer cells (HT-1376) secrete detectable amounts of MIF protein.”

2- Page 11. MIF localization in normal human bladder. This is supposed to be presented in figures 1A and 1B. However, the legend of figure 1 on page 30 indicates – Effect of Treatments on HT-1376 cell proliferation.
It seems that the legend of figure 1 is missing. In addition, it seems that the first legend belongs to figure 2.

3- Histological figures 1A and 1B. It seems that MIF immuno reactivity is localized to umbrella cells. Is this correct? I could not find the nuclear localization of the stain. Will this figures be publish in color? If not, please add additional arrows on Figure 1B to indicate the localization of your in situ results.

4- Page 11. The rationale for using the minimum effective concentration instead of the effective concentration 50% was not clear. It seems that for anti-MF it was difficult to determine the EC50 since there was a plateau. Nevertheless, is this the reason for a modest reduction in cell numbers? Have the authors tried to determine if this was reflected by a significant reduction in the proliferation index?

5- Page 12. Is caspase-3 the only pathway for urothelial cell apoptosis?

6- Cytokine release (Tables 1A and 1B). How sure are the authors that those proteins are secreted and not a result of cell death and release of cytoplasmic contends? Some of the results on table 1 A and B are presented as ratio. I assume that the ratio was treatment/control. The visualization of this table is very difficult. It is recommended that an asterisk should be placed next to the numbers that represent 2.0 fold and greater down regulation or that an invert ratio (control/treatment) should be used in order to facilitate the visualization of fold-variation in protein levels. Alternatively, protein levels can be expressed as % of control.

7- Tables 1A and 1B- The results do not give any idea in terms of variation. As stated on page 14, conditioned medium from all HT-1376 controls (untreated, non-specific IgG, and sense) were analyzed and although there was no difference in protein ratios in different controls, this result would give an idea of the variation of the experiment.

8- Figure 5, please add the MW next to each lane.

9- Figures 5B and 5D should have a clear label indicating which protein is being described.

Discretionary Revisions (which the author can choose to ignore)

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests:

None