Author's response to reviews

Title: Null Mutation for Macrophage Migration Inhibitory Factor (MIF) Is Associated with Less Aggressive Bladder Cancer in Mice

Authors:

John A Taylor III (jtaylor@uchc.edu)
George A Kuchel (kuchel@nso1.uchc.edu)
Poornima Hegde (hegde@uchc.edu)
Olga S Voznesensky (vozneseskk@nso1.uchc.edu)
Kevin Claffey (claffey@nso2.uchc.edu)
John Tsimikas (tsimikas@uchc.edu)
Lin Leng (Lin.Leng@Yale.edu)
Richard Bucala (Richard.Bucala@Yale.edu)
Carol Pilbeam (pilbeam@nso.uchc.edu)

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Author's response to reviews: see over
Dr. John A. Taylor, III, MD  
Assistant Professor of Surgery  
Division of Urology  
University of Connecticut Health Center  
Farmington, CT 06030

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To the Editor;

Thank you for the review of our manuscript entitled “Null Mutation for Macrophage Migration Inhibitory Factor (MIF) Is Associated with Less Aggressive Bladder Cancer in Mice.” We have made the requested format changes to the manuscript and addressed the reviewers concerns as discussed below.

Reviewer 1: Pathological observation is purely qualitative. Need quantitative or semi-quantitative date, as described at methods section. How about the number of CIS and cancerous lesions, width, depth and etc? Similarly, they should provide the number of cancerous lesions with which they studied the muscle invasion. The tumors that develop in this model are bulky and occupy the majority of the bladder lumen. Therefore each tumor is representative of each bladder and one tumor equals one bladder. We have added text to emphasize the quantitative difference between the invasive and non-invasive tumors (page 9, paragraph 1, line 14). We agree that in bladder cancer the number and size of lesions is important for superficial, low-grade disease. However, prognosis rests on the most aggressive or highest stage tumor regardless of number or size. Our model is one of high-grade disease and we saw no difference in the timing of cancer presentation or the aggressiveness (grade) of the tumors between the wild-type and MIF knock-out mice. All tumors were bulky, occupying the majority of the bladder lumen with the only pathologic difference being stage – the most prognostic finding. We have clarified this in the text (page 9, paragraph 1, lines 8-11).

At result section, description of “a linear regression model” should be shortened, because it has little impact in the study. This section has been shortened as suggested.

How about the difference in MIF staining intensity among normal, metaplasia, atypia, CCIS and carcinoma, and between non-cancerous and cancerous lesions in the same bladder? We saw no difference in MIF staining intensity or nuclear translocation in the normal and metaplastic urothelium or CIS. Increased staining and nuclear translocation were only seen when invasive cancer was present. The rare uninvolved urothlum in the bladders with tumor showed the same findings as in the control bladders (cytoplasmic with rare nuclear staining). This has been clarified in the text (page 9, paragraph 2, lines 3-5).
Reviewer 2: In this study, treated with BBN, muscle invasion was present in all MIF WT bladders, whereas MIF KO bladders had lower tumor stage with no invasion into muscle layers. In terms of cell growth, apoptosis, or angiogenesis, how do the authors speculate why the absence of MIF do not impair tumor development but is associated with the absence of tumor invasion especially into muscle. It is unclear what is the exact mechanism involved in muscle invasion. It is thought that MIF secretion by tumor cells acts locally to increase tumor cell viability, by increasing proliferation and decreasing apoptosis. However, secreted MIF may have a pro-apoptotic effect on adjacent bladder smooth muscle cells, thus preparing the way for invasion. MIF has also been shown to increase angiogenesis and to increase MMPs, which may enhance tumor cell invasion. On page 13, paragraphs 1 & 2 we discuss possible mechanisms that may play a role in this process such as increased MMP expression, increased invasive capacity and pro-apoptotic effect on bladder smooth muscle cells.

As the authors mentioned, the role of MIF in cell proliferation, angiogenesis, or tumorigenesis remains unclear and seems very interesting. Do the authors have any data about the influence of MIF deficiency on MAPK, ERK, or PI3K pathways? The role of each of these in MIF signaling has been reported in the literature. MIF action appears to result in sustained activation of the MAPK/ERK pathway and has recently been shown to promote survival in fibroblasts via a phosphoinositide-3-kinase (PI3K)/Akt signaling pathway. Text reflecting this has been added to the discussion (page 12, paragraph 1, lines 6-13). Although we did not examine these factors in this model we plan to include these in an expanded study.

When you think about cell proliferation, Cyclin D1 for instance is a critical regulator involved cell cycle progression which controls cell proliferation, and did the authors study the expression or something of this factor under MIF influence? This is an excellent suggestion. MIF has been shown to increase Cyclin D1 transcription. Text reflecting this was added to the discussion (page 12, paragraph 1, lines 9-11). We did not evaluate proliferation in this model but do have studies underway in bladder cancer cell lines.

In future, how are the authors planning to determine the role of MIF in human bladder cancer? How about the possibility of MIF as a specific marker for invasive bladder cancer? One of our goals to take our findings from an animal model to human bladder cancer. We have altered Figure 2 to include some preliminary data on MIF staining and nuclear translocation showing lack of nuclear expression in benign human urothelium and marked nuclear staining in a human specimen of invasive cancer. We are considering the possibility that increased nuclear translocation or increased MIF secretion might correspond with invasion. If we can replicate our findings in cell culture and human specimens, we would then begin to look at MIF polymorphisms which have been reported as prognostic in several disease processes.

Other general changes or concerns include;
Our previous publication: Am J Physiol Renal Physiol 2006;291:f1343, is listed as reference 19 and has been included with the resubmission documents.
Page 4, paragraph 1, line 1 – numbers changed to reflect most recent figures on bladder cancer.

We hope that these modifications address the concerns of the editorial staff and reviewers. Thank you again for considering our manuscript for publication. We look forward to hearing from you.

Sincerely,

Dr. John A. Taylor, III, MD